1

Microscopy of soft materials

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1.1 Introduction

"Soft materials" is a loose term that applies to a wide variety of systems we encounter in our everyday experience, including:

- Colloids, which are microscopic solid particles in a liquid. Examples include toothpaste, paint, and ink.
- Emulsions, which are liquid droplets in another immiscible liquid, for example milk and mayonnaise. Typically a surfactant (soap) molecule or protein is added to prevent the droplets from coalescing.
- Foams, which are air bubbles in a liquid. Shaving cream is a common example.
- Sand, composed of large solid particles in vacuum, air, or a liquid; examples of the latter include quicksand and saturated wet sand at the beach.
- Gels are cross-linked polymers such as gelatin, or sticky colloidal particles. Usually the components of a gel (the polymers or particles) are at low concentration, but the gel still is elastic-like due to strong attractive forces between the gel components.

One common feature to all of these materials is that they are all comprised of objects of size 10 nm–1 mm; that is, objects much larger than atoms. In fact, it is these length scales that gives them their softness, as a typical elastic modulus characterizing these sorts of materials is $k_B T/a^3$, where k_B is Boltzmann's constant, T is the absolute temperature, and a is the size of the objects the material is made from [1]. For example, a could be the radius of a colloidal particle or of a sand grain, or in a conventional crystalline solid a would be the lattice spacing. For soft materials such as those listed above, a is much larger than the lattice spacing of a crystalline solid, resulting in a much reduced elastic modulus. This then is why soft materials are "soft."

Experimental and Computational Techniques in Soft Condensed Matter Physics, ed. Jeffrey Olafsen.
Published by Cambridge University Press.

While grains of sand are large enough to be seen with the naked eye, smaller objects, such as micron-diameter colloidal particles or emulsion droplets, are sufficiently large enough that they are easily viewed with optical microscopy. For this reason, microscopy has become an important tool for studying the structure of these types of samples, for example how the bubbles in a foam are arranged.

Along with the spatial scales, the temporal scales of these soft systems are often compatible with conventional video microscopy. For example, consider food products such as mayonnaise or peanut butter. These are somewhat solid-like, in that one can imagine a glop of peanut butter but not a puddle of peanut butter; however, they are also fairly easy to spread with a knife. The speed of spreading with a knife is set by human time scales. For example, a knife moved with velocity $v = 10$ cm/s spreading peanut butter with thickness $h = 4$ mm results in a strain rate given by $\dot{\gamma} = v/h = 25\ \mathrm{s}^{-1}$. Thus, to understand what happens to the peanut butter microscopically when it is deformed by the knife, one might wish to take 25 images per second, which is easily achieved with inexpensive video cameras that are straightforward to connect to a microscope.

Another relevant time scale is set by diffusion. Very small particles undergo Brownian motion due to thermal fluctuations. The diffusion constant D for a sphere of radius a is given by the Stokes–Einstein–Sutherland formula:

$$D = k_B T/6\pi\eta a \tag{1.1}$$

where η is the liquid viscosity [2, 3]. D then can be used to quantify the particle motion as follows. The motion in the x direction is equally likely to be left or right, so the mean displacement $\langle \Delta x \rangle = 0$, where the angle brackets indicate an average over many different particles. However, the mean square displacement will not be zero, but instead will be proportional to the time Δt over which the displacements are measured:

$$\langle \Delta x^2 \rangle = 2D\Delta t; \tag{1.2}$$

thus a larger value for D results in faster motion. $\langle \Delta x^2 \rangle$ is often called the variance. If you can imagine injecting a tiny blob of dye molecules at a single point, then the expanding cloud of dye has a characteristic size $\sqrt{\langle \Delta x^2 \rangle}$.

Using the mean square displacement, we can estimate the Brownian time scale τ_B as the time a typical particle takes to diffuse its own size as:

$$\tau_B \equiv a^2/2D = 3\pi\eta a^3/k_B T. \tag{1.3}$$

For a particle of diameter $2a = 1\ \mu$m in water ($\eta = 1$ mPa·s) at room temperature, $\tau_B \approx 1$ s. Again, this motion is easy to study with a conventional video camera and a microscope. Of course, particles that are ten times smaller move 1000 times

faster, by Equation (1.3); nonetheless, many systems of interest have micron-sized components.

This chapter will discuss several types of optical microscopy, although it will not provide a complete survey of the variety of microscopy techniques; the interested reader should consult reference [4]. Likewise, several representative applications of microscopy will be presented, although this will not be an exhaustive review; for more comprehensive review of articles of the applicability of microscopy to soft matter experiments, see references [5–8].

1.2 Video microscopy

Conventional optical microscopes are powerful, highly sophisticated instruments, which are nonetheless straightforward to use [4]. They are common in biology and biochemistry laboratories, and therefore it is easy to borrow time on one if you do not wish to purchase a microscope. For data acquisition, it is simple to attach a camera to the microscope, whether it be a conventional "snapshot" camera or a CCD video camera. The latter is now more common, and well-suited for studying samples which move or change. The output of the CCD video camera is usually attached to a frame grabber card in a computer, so that data are saved digitally, although a conventional VCR (video cassette recorder) can also be used.

There are several types of optical microscopy, and frequently the same microscope can be used in different ways by making slight modifications. The simplest technique is termed *brightfield microscopy*. Here, the light source is focused by a lens onto the sample, and the objective lens on the other side of the sample collects the light, allowing the user to see an image of the sample, for example the left image in Figure 1.1. Note that microscopes can be either "upright," where the objective lens is above the sample and the light source below, or "inverted," where the situation is reversed. Upright configurations are good for samples that "float," such as Langmuir–Blodgett films, which are layers of surfactant molecules on the surface of a water bath [9, 10] (see also Chapter 4). Inverted configurations are useful for samples that "sink," such as suspensions of dense particles. In some cases, inverted microscopes are also useful as the light source can be moved far above the sample, and other instrumentation then placed above the objective.

In brightfield microscopy, the image contrast can be due to components in the sample which absorb light (dyes, for example) or variations in the index of refraction of the sample. Variations in the refractive index are common, such as between oil and water in an emulsion; both oil and water are transparent, but the differences in the index of refraction allow the oil droplets to be seen. A good example of this effect is milk, which is white not because it contains white components, but because the variations in the index of refraction between the water and the milk fat scatter light randomly, resulting in a white appearance. A similar argument explains why

snow is white, despite the transparency of ice; it is the reflection and refraction at the ice/air interfaces within the snow.

A second common method is *fluorescence microscopy*. Here, short-wavelength light is sent in, typically through the objective. Fluorescent molecules in the sample absorb this light, and radiate slightly longer wavelength (lower energy) light, which is collected through the objective. Special filters and mirrors are used to direct the light appropriately from the light source to the sample, and from the sample to the camera. In particular, a "dichromatic" (or "dichroic") mirror reflects the excitation light onto the sample, but allows the emitted light to pass through to the camera or microscope eyepiece. The advantage of fluorescence microscopy is that the dye can be placed in specific parts of the sample, such as in the solid particles of a colloidal suspension, or even in the surfactant molecules stabilizing emulsion droplets. This makes it easier to distinguish different sample components.

Fluorescence microscopy has one significant limitation: photobleaching. After dye molecules absorb the excitation light, but before they emit light, they can chemically react with oxygen present in the sample to form a non-fluorescent molecule. This only happens when they are excited, so photobleaching happens in direct proportion to the illumination light (with the limitation that once all the dye molecules are excited, they have reached saturation). Thus a sample can sit stably for a long time in the dark, but the photobleaching starts precisely when the sample is observed, that is when the excitation light is turned on (although, this only affects the local region illuminated by the microscope). Photobleaching manifests itself as the image becoming gradually darker. In some cases, photobleaching can be delayed by adding chemicals such as propyl gallate to the sample, which will bind to the free oxygen in the sample. This trick is limited to samples which are compatible with such chemicals, generally restricting the use of these chemicals to nonbiological samples. In other cases, photobleaching can be useful for studying local diffusion in samples, a technique known as "fluorescent recovery after photobleaching" [11]. Intense light is used to photobleach a region of the sample, and then low-intensity light is used to monitor the recovery of fluorescence as nonbleached dye molecules diffuse back into the region. With this method, the diffusivity of the dye molecules can be measured.

There are other common types of optical microscopy such as darkfield microscopy, phase contrast microscopy, and differential interference contrast microscopy (see the right image in Figure 1.1). These are modifications of bright-field microscopy and are able to enhance the contrast from very slight differences in the index of refraction. These techniques are more often used in biology, where one might wish to study a cell, filled with water and biopolymers, immersed in a cell culture medium that also is primarily water. These are less often used for soft condensed matter; for more details, see reference [4].

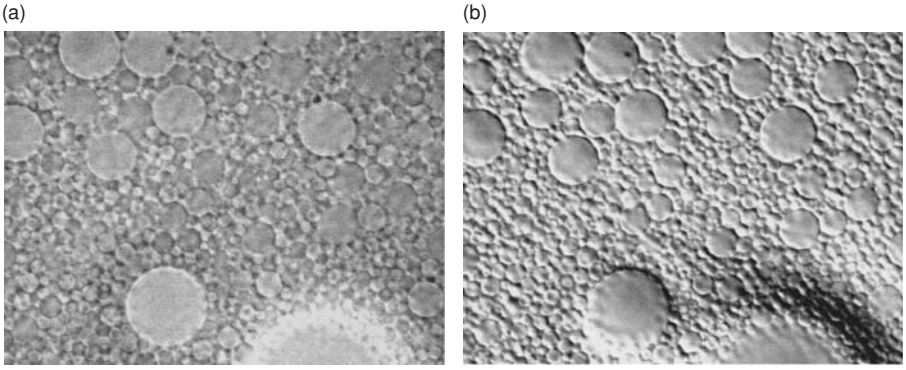

Figure 1.1 (a) Image of an oil-in-water emulsion taken using brightfield microscopy. The picture is ∼60 μm wide. (b) Image of the same field of view taken using differential interference contrast microscopy. This form of microscopy produces a fictitious three-dimensional appearance; in reality, these are the cross-sections of spherical droplets. *Note:* the contrast in both pictures has been enhanced for better appearance.

1.3 Confocal microscopy

A confocal microscope is a laser scanned optical microscope. This is a fluorescent technique; the laser light is used to excite fluorescence in dye added to a sample. Typically, the laser beam is reflected off two scanning mirrors that raster the beam in the x and y directions on the sample. Any resulting fluorescent light is sent back through the microscope, and becomes descanned by the same mirrors. A dichroic mirror is used to then direct the fluorescent light onto a detector, usually a photomultiplier tube.

One additional modification is necessary to make a confocal microscope: before reaching the detector, the fluorescent light is focused onto a screen with a pinhole. All of the light from the focal point of the microscope passes through the pinhole, while any out-of-focus fluorescent light is blocked by this screen. This is crucial for viewing samples which may be full of fluorescent objects. Figure 1.2 shows excitation light being focused through a sample, and clearly the highest intensity of the excitation light is at the focus of the lens. However, the weaker out-of-focus light still excites fluorescence in other layers of the sample. While this emitted light is much weaker (in proportion to the excitation light), there is a large volume where this out-of-focus emitted light emanates. The pinhole filters out most of this out-of-focus light, allowing a strong and clean signal to come from the in-focus region. The pinhole is *conjugate* to the *focal* point of the lens, meaning that a point in focus at the focal point of the objective lens is re-imaged onto the pinhole, and this is the origin of the term "confocal." Intriguingly, the confocal microscope was

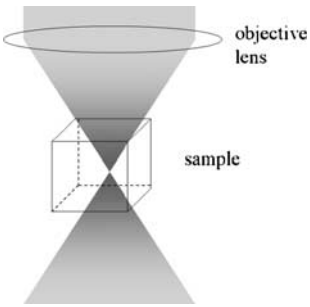

Figure 1.2 Sketch of light being focused by an objective lens. While the intensity is highest at the focal point, other portions of the sample are illuminated as well.

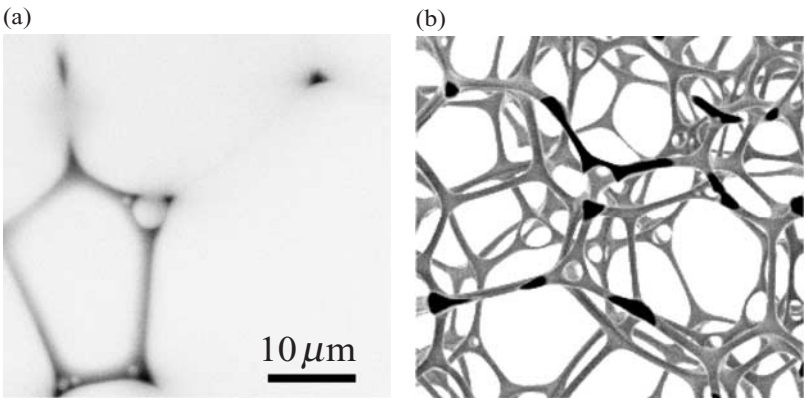

Figure 1.3 Images from confocal microscopy. (a) 2D image of a foam. Because of the narrow depth of focus of confocal microscopy, only foam channels that are close to parallel to the field of view are visible. At the upper right, a channel perpendicular to the field of view can be seen as a small black triangle. The depth of focus of this image is approximately 0.5 μm. (b) 3D image of a foam, 100 μm wide. (Images from Doug Wise and Eric R. Weeks.)

invented by Marvin Minsky in 1955, who is much better known for his work in artificial intelligence [12, 13].

This ability to reject out-of-focus fluorescent light directly results in the main strength of confocal microscopy, the ability to take three-dimensional pictures of samples. By rejecting out-of-focus light, a crisp two-dimensional image can be obtained, as shown in Figure 1.3(a). The sample (or objective lens) can be moved so as to focus at a different height z within the sample, and a new 2D image obtained. By collecting a stack of 2D images at different heights z, a 3D image is built up, as shown in Figure 1.3(b).

The time to scan one 2D image can range from 10 ms to several seconds, depending on the details of the confocal microscope and the desired image size and quality. For faster rates one typically substitutes an Acoustic-Optical Deflector

("AOD") for one of the scanning mirrors. This uses a radio-frequency sound wave to set up a standing density wave pattern in a crystal. The standing wave acts as a diffraction grating and steers the laser light depending on the wavelength of the standing wave, which is controlled by the sound wave. Because there are no moving parts, the AOD is faster than a scanning mirror. However, because the diffraction grating behavior is dependent on the wavelength of the diffracted light, the fluorescent light (being a longer wavelength than the excitation light, and not monochromatic) cannot be descanned by the AOD. Thus, AOD-based confocal microscopes replace the confocal pinhole with a confocal slit, with some slight loss of optical performance.

Another high-speed confocal microscopy technique is the Nipkow disk confocal microscope. This uses a spinning disk with many pinholes in it, such that some fraction of the field of view is illuminated at any given moment. As the disk spins, the entire field of view is scanned. The collected light is imaged by a standard video camera rather than a photomultiplier tube, and thus by using different cameras, the technique can be adapted to different conditions (low light levels, higher speed, etc.) Given that there are no moving mirrors, the scanning speed can be quite fast, 100 images/s or more depending on the camera choice. The drawback is that the resolution is not as good, as more out-of-focus light returns through the pinholes.

1.4 Strengths, weaknesses, and tradeoffs

1.4.1 Strengths of optical microscopy

Like the other methods described in this book, optical microscopy has strengths and weaknesses. One strength is the ability to visualize the heterogeneous structure. Often, complex fluids are spatially heterogeneous (for example, colloidal gels such as the one shown in Figure 1.4), and it may be desirable to understand this structure. A second strength is the ability to distinguish different features of the material, for example by using fluorescence techniques and adding different dyes to different regions. A third strength is that the data provided by video microscopy are fairly straightforward to understand; directly imaging what is in a sample can often be easier to interpret than more indirect measurements. A fourth strength is the ability to understand local properties. An example of this is studying how granular particles pack next to a wall, and comparing that with the packing in the interior. Other examples will be given in Section 1.6.

1.4.2 Weaknesses of optical microscopy

A weakness of video microscopy is the necessity to prepare the sample to be compatible with microscopy. Often this means matching the index of refraction of the components. For example, consider a dry sample of small glass spheres,

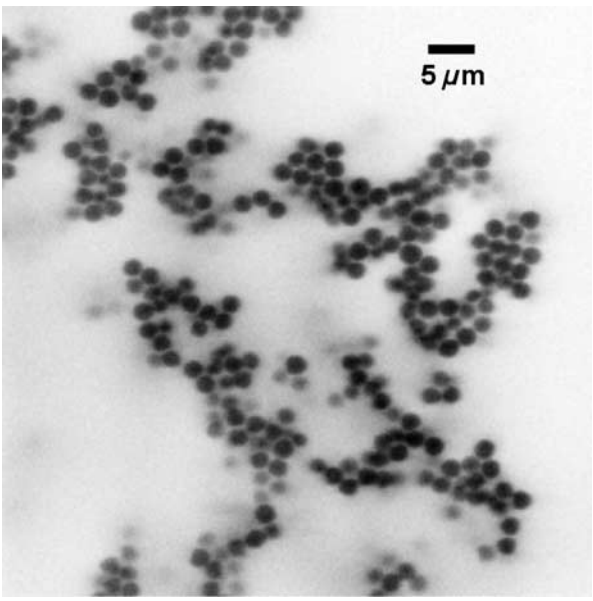

Figure 1.4 Image of a colloidal gel, taken with confocal microscopy. The particles are 2 μm diameter and aggregate due to the depletion force which is caused by adding small polymers to the solvent [14]. Image from Gary Hunter and Eric R. Weeks. See Chapter 3 for more information about colloidal gels.

perhaps each 50 μm diameter. These are of the right length scale for microscopy, but each sphere acts like a small lens and scatters light. Thus, this sample will look like white powder, for the same reason as the milk and snow examples discussed above. Microscopy would only be able to see the first layer of particles clearly. To better observe this sample, you would need to add an index-matching fluid. However, then you would be studying a wet sample, and the properties might be much different [15–19].

A second weakness of video microscopy is the lack of averaging. This shortcoming is complementary to the strength of visualizing spatially heterogeneous structures: while a good picture is gained of the local structure, it might be necessary to examine a large number of different regions to get a true picture of the average structure. For example, consider the case of colloidal crystallization. A sample of monodisperse hard sphere-like colloidal particles can form crystals, usually hexagonal-close-packed, as shown in Figure 1.5. This occurs at volume fractions $\phi > 0.494$, for reasons due to maximizing entropy by improving the local packing; for a fuller description, see references [1, 20]. Microscopy can be used to study the nucleation of these crystals, but is limited to the crystals that happen to nucleate within the field of view [20, 21]. Direct observation with microscopy can thus determine the specific shapes of a crystal nucleus. On the other hand,

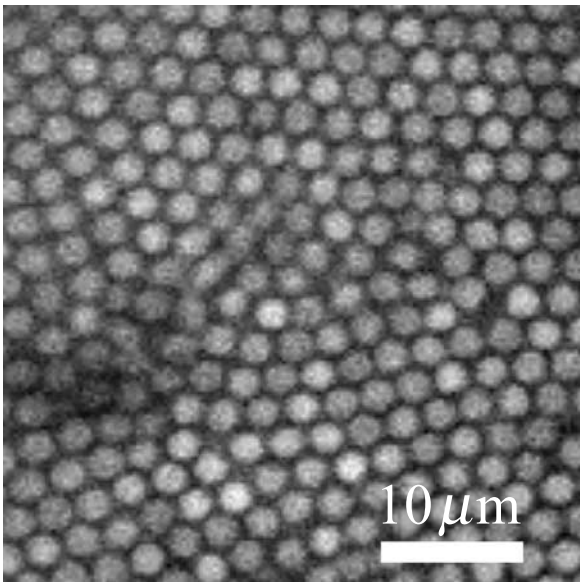

Figure 1.5 Confocal microscope image of a colloidal crystal. The black patch at the lower left is due to out-of-focus particles, likely because of a crystalline defect. A grain boundary is seen running through the middle of the image. The particles have a radius $a = 1.18$ μm and the sample is otherwise similar to those described in reference [20].

a spatially averaging technique like light scattering is better able to measure the average nucleation rate, but is unable to determine nuclei shapes [22].

1.4.3 Tradeoffs when doing optical microscopy

The speed of image acquisition can be either a strength or weakness, depending on the experiment. Video cameras typically acquire images at 30 frames per second (fps). Interlaced video cameras acquire an image alternately from the odd and even rows of pixels, and thus acquire half-images at 60 half-frames per second, which can be useful in some cases [23]. Confocal microscopes, as noted above, have speeds ranging from 1 fps to 30 fps, depending on the hardware. It is possible to use faster cameras, but the tradeoff is then the need to have an illumination level sufficient for the camera. Higher illumination levels (required for faster imaging) will often result in substantial heating of the sample. For fluorescent samples, higher illumination levels cause faster photobleaching.

For confocal microscopy, the main way to achieve faster speeds is to decrease the field of view and number of pixels in the image, so that the scanning optics have shorter distances to cover. One can then maintain the same light levels and the same photobleaching rate as with the slower scanning speed over a larger field

of view. It is, of course, harder to take three-dimensional images very quickly; the limitation is how fast the objective can be moved to scan in the z direction. Scanning in the z direction is often achieved by attaching the microscope objective to a fast piezo-electric transducer, which is in turn attached to the microscope. In this way, 3D images of reasonable size can be acquired at speeds of more than 1 image/s, with faster rates possible for thinner image stacks (thinner in z).

One straightforward tradeoff is the optical resolution as compared to the field of view. Higher magnification lenses have better optical resolution, but at the price of looking at a smaller region within the sample. However, to understand this tradeoff one first needs to understand the relevant terms:

- **Magnification**: Technically this is defined as the apparent angular extent of the image as seen by the eye, compared to the actual angular extent of the object if it was at a reference distance of 25 cm from the eye. In practice, magnification is not a crucial parameter. Any image can be magnified as much as desired by projecting it onto a big screen. Objective lenses typically range from $5\times$ to $100\times$ magnification, and the rest of the microscope optics typically provide an additional factor of $10\times$.
- **Field of view**: More directly useful than the magnification, this is the region within the sample that is viewed. For the highest magnification lenses, the field of view can be as small as $50 \times 50\ \mu\text{m}^2$. Field of view also relates to the camera and the optics attaching the camera to the microscope. Typically the field of view as seen by the camera is a quarter of the area seen through the microscope eyepieces.
- **Size of image in pixels**: This depends on the camera. Having more pixels over the same field of view is often helpful, although it requires more room to store the data on a computer. Note, however, that switching to a camera with more pixels does not increase the field of view, which is set by optics. However, the physical size of the CCD chip within a camera can impact the field of view.
- **Resolution**: The optical resolution is set by diffraction effects, quantified by Rayleigh's criterion, and is tied with the quality of the optics and the wavelength of light used to view the sample [4]. The best (smallest) resolution of optical microscopes is about 200 nm. The optical resolution quantifies the ability to distinguish two closely spaced objects. For example, a typical test pattern is a grid of lines, and if the grid spacing is smaller than the resolution, no lines will be seen. Another way to think about resolution is to consider the fluorescence microscopy image of a small fluorescent molecule. The size of the molecule is much smaller than the resolution, but the light emitted from the molecule can still be seen. However, rather than appearing as an extremely sharp point of light, the molecule appears as a fuzzy round spot with a diameter equal to the optical