Imaging and other techniques
Seeing is believing: the role of microscopy in determining morphology and kinetics of colloids

Editorial overview
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Abbreviations
AFM atomic force microscopy
BAM Brewster angle optical microscopy
ESEM environmental scanning electron microscopy
HRTEM high resolution transmission electron microscopy

The combined goals of microscopy techniques when applied to any material are to improve resolution to the maximum extent possible, while minimizing any artefacts associated with sample preparation and imaging [1]. A new goal is to be able to image kinetic events while they are happening in realistic environments with a minimum of sample preparation or alteration [2,3]. The reviews presented here were chosen to demonstrate the state of the art in achieving these goals when microscopy is used to determine structure, kinetics and mechanisms in colloid science. We have chosen to present illustrations from four major types of microscopy—the first two that are readily accessible to most university and industrial researchers: high resolution transmission electron microscopy (HRTEM) and atomic force microscopy (AFM).

Hudson (pp 125–130) presents the application of HRTEM to the study of liquid crystalline polymers. HRTEM has proven quite valuable over the past few years in determining the structures formed by copolymers, polymer blends, and related materials. The main difficulties have been overcoming the sensitivity of these materials to the electron beam by developing appropriate sample preparation methods and so called ‘low dose’ imaging conditions. New methods of computer aided image processing have allowed even more information to be obtained from a given image. Schwartz (pp 131–136) reviews the extention of AFM to in situ kinetic studies of monolayer growth and melting. The AFM is unique in that molecular resolution images can be obtained under realistic conditions—including imaging in the solvents used to prepare self-assembled monolayers, or even during melting of thin films (see Figure 3 of Schwartz review).

The second two reviews deal with two relatively new microscopy techniques that have been developed over the past decade, but should become much more widely used in the near future: environmental scanning electron microscopy (ESEM) and Brewster angle optical microscopy (BAM). Möbius (pp 137–142), one of the co-developers of BAM [4,5], presents an overview of the principles and possibilities of BAM, which can be thought of as the imaging version of the more commonly used method of ellipsometry. BAM takes advantage of the extreme sensitivity of the Brewster angle, which is the angle at which light is totally reflected from an interface, on the index of refraction of the materials at that interface. With BAM, even sub-monolayer modifications of an interface can be detected and quantified. BAM is an alternative to the more commonly used fluorescence microscopy, in which a dye is added to the material at the interface that is preferentially located in different phases [6,7]. With BAM, no additional dye is needed, and any complications in the phase behavior, kinetics, or morphology due to the dye can be identified and eliminated. Old instruments can also take on new life, such as the environmental SEM, which uses a series of differential pumping chambers to allow for near ambient water vapor pressure to be maintained at the sample, while allowing the electron gun to operate at high vacuum. The ESEM can then provide the outstanding three-dimensional, sub-micron resolution images of the SEM, but on hydrated samples. Donald (pp 143-147) gives a brief overview of the instrument and operating conditions and some of the early successful applications of ESEM to colloid science.

Microscopy is still somewhat underutilized in the field of colloid science relative to scattering and spectroscopy techniques, and this is probably due to well-publicized artefactual images, the high cost of developing a microscopy program from scratch, and the technical difficulties in making and imaging samples containing liquids or other dynamic materials. Imaging, however, presents structure and all its manifestations in the most direct and easily accessible way possible—in real space and with a minimum of interpretation. A real space image can also be much more easily Fourier transformed to provide the complementary ‘diffraction’ pattern than can the Fourier information of an inverted light, X-ray or neutron scattering pattern to give a ‘real’ image [1]. In addition to the ‘bulk’ or average structures, defects can play an enormous role in the ultimate properties of materials, and are quite often only accessible via microscopy techniques.
(2); see Hudson’s and Schwartz’s reviews). Microscopy provides very localized information, without the averaging inherent to scattering methods. In the study of defects, this is often an advantage in that the defect can be identified and characterized at high resolution (2); see Hudson’s and Schwartz’s reviews). Nevertheless, care must be taken not to interpret a limited number of images at being truly representative of the bulk structure. The same warning should also be made to investigators relying exclusively on indirect scattering data—the interpretation of scattering data is model dependent and limited by the mathematical or physical sophistication of the interpreter; such models can invariably benefit from real space imaging (2,3,8). The optimum path is to combine direct imaging with scattering, spectroscopy, and other physical and chemical characterization techniques to provide the most comprehensive picture possible (11–3; see Schwartz’s review).

Figure 1

![Image of monolayer of dipalmitoylphosphatidylglycerol with 10% of a peptide based on the lung surfactant specific protein SP-B at 37°C on pure water showing the bright, fluid phase network segregating the darker, condensed phase domains. The inset is an AFM image of one of the interstitial regions transferred to a mica substrate (10). The height difference between the protein rich network phase and the surrounding condensed domains is 5 nm (inset bar=2 μm). The combined techniques give both dynamic and three dimensional information which is otherwise difficult to obtain.](image)

Monolayer of dipalmitoylphosphatidylglycerol with 10% of a peptide based on the lung surfactant specific protein SP-B at 37°C on pure water showing the bright, fluid phase network segregating the darker, condensed phase domains. The inset is an AFM image of one of the interstitial regions transferred to a mica substrate (10). The height difference between the protein rich network phase and the surrounding condensed domains is 5 nm (inset bar=2 μm). The combined techniques give both dynamic and three dimensional information which is otherwise difficult to obtain.

Combining different types of microscopy techniques can also confer distinct advantages in minimizing artefacts, correlating information at widely varying resolutions, and improving sample preparations [8–10]. Woodward and Zasadzinski [8] combined scanning tunnelling microscopy with freeze-fracture electron microscopy to develop three-dimensional images of ripple phases of phosphatidylcholines in excess water. Lee et al. [10] have combined optical fluorescence microscopy with a novel inverted Langmuir–Schaefer monolayer deposition to correlate domains at the air–water interface with high resolution AFM images of the same domain after transfer to mica or silicon substrates (see Figure 1). The fluorescence images of monolayers of dipalmitoylphosphatidylglycerol, lung surfactant protein SP-B, and a small fraction of fluorescent dye show an unusual bright (corresponding to a disordered phase) network surrounding islands of a more ordered phase. Transfer of these domains to a mica substrate showed that the network phase was substantially thicker (about 5 nm) than the condensed phase domain, suggesting that the protein was preferentially located in the network phase [9,10].

References