ADVANCED MATERIALS

Research News

Imaging Individual Particles in Concentrated Colloidal Dispersions by Confocal Scanning Light Microscopy**

By Alfons van Blaaderen*

1. Introduction

Interest in the study of concentrated, or highly interacting, colloidal dispersions stems not only from the wide range of technological applications (e.g. paints, ceramic materials, electro-rheological fluids) of these systems, but also from their use as model systems to investigate fundamental aspects of chemistry and physics. Dispersions are such good model systems because it can be shown that the thermodynamic properties of a dispersion of colloidal particles is formally the same as that of an assembly of atoms or molecules.[1] The analogue of the inter-atomic potential is the "potential of mean force", which in many cases can be approximated by an effective pair potential between two colloidal particles. By studying model dispersions, therefore, physical theories on, for example, the structure of liquids or crystals can be tested.[1-3]

Nowadays, most of the structural and dynamic information on concentrated dispersions is obtained by light, neutron or X-ray scattering.[1, 3] One of the main disadvantages of these scattering techniques is that the measured quantity is necessarily an averaged bulk property, whereas in many cases local information is just as important. For instance, the strength of a ceramic material depends to an important extent on the nature and concentration of defects that are present in the particle packing. Also, the ability of an electro-rheological fluid to be transformed into a gel-like state through the application of an electric field depends on the formation of string-like particle structures. The presence of such structures was demonstrated using microscopy.[4] Furthermore, complicated structures of binary colloidal crystals were determined first with the direct imaging techniques electron microscopy[5] and light microscopy.[6]

Despite its high resolution, however, electron microscopy is not well suited to the study of dispersions because of the necessity to use vacuum. Conventional light microscopy has the disadvantage that the resolution is limited and that out-of-focus information contributes to the image. This last fact limits the range of observation to low volume fractions of particles and to only a few layers of particles away from the cover glass.[6, 7] As will be explained, confocal scanning microscopy combats most of the above-mentioned limitations.

2. Confocal Scanning Light Microscopy

The principle upon which the advantageous properties of confocal scanning microscopy rest has been known for quite sometime.[8] However, it was not until lasers and low-cost computer imaging equipment and data processing systems became available that confocal imaging became popular. Nowadays, a host of commercial instruments can be bought.

In essence, confocal microscopy does not image the entire object field simultaneously, but rather limits its field of view to just one diffraction-limited point at a time. The price that has to be paid is, of course, that now the entire image has to be built by scanning the beam over the object (or vice versa, moving the object relative to the beam). The name *confocal* stems from the fact that not only the objective lens probes the specimen with a finely focused spot of usually laser light, but that also the collector lens images the reflected or fluorescent light on a point detector. (A confocal microscope can also be operated in transmission.) Thus, the point source illuminates only a very small portion of the object, and the point detector ensures that only the light from the same small area is detected.

The double imaging of a diffraction-limited spot leads to increased resolution compared to conventional light microscopy and, even more importantly, the use of both a point source and a point detector results in a powerful depth discrimination. This depth discrimination or optical sectioning is due to the detection of only the light that comes from the focal region of the confocal lens. Typical values for the lateral or in-plane resolution are 200×200 nm and 650 nm for the axial resolution. Here, the use of a lens with numerical aperture (N.A.) of 1.3 is assumed together with 488 nm excitation light and a fluorescence emission that is not too far

[*] Dr. A. van Blaaderen
Van 't Hoff Laboratory, University of Utrecht
Padualaan 8, 3584 CH Utrecht (The Netherlands)

[**] A. Imhof and N. A. M. Verhaegh are thanked for their help with the synthesis and W. Hage (Netherlands Institute for Developmental Biology, Utrecht) is thanked for his help with the confocal microscopy.

  0935-9648/93/0101-0052 $ 3.50+.25/0

from this wavelength.[8] With beam scanning it typically takes about 1 s to scan an image containing 512 × 768 pixels.

The strong optical sectioning together with digital data storage and processing make it possible to use a stack of successive optical sections to obtain 3-D structural information. The possibilities of confocal microscopy have already been shown in biology, biochemistry and physiology.[8] We expect, however, an important role for this technique in colloid and materials science as well.

A good example of the importance of using confocal microscopy has recently been presented.[9] In this paper Yoshida et al. conclude that the formation of colloidal crystals from polystyrene latex spheres in water is strongly affected by the glass–suspension interface. Before using (brightfield) confocal microscopy, these authors could only investigate particles a few micrometers behind the cover glass and could only guess at the extent of the role of the glass.

Bremer used confocal fluorescence microscopy to study the fractal dimensionality of a casein gel in water.[10] However, the particles that formed the gel had a radius of 80 nm and could not be resolved separately. In the next section we show how, by using new, specially prepared, fluorescent silica particles, the advantages of the confocal technique can be fully exploited.

3. CSLM Graphs of Concentrated Dispersions of Fluorescent Silica Spheres

Recently, we succeeded in placing silane coupling agents covalently inside monodisperse silica spheres.[11] Using this method, silica spheres with a fluorescent organo-silica core and a non-fluorescent silica shell can be made by attaching a dye to the coupling agent. Because the dye is far away from the particle surface, it cannot influence the inter-particle potential. Further, the non-fluorescing layer makes it easier to distinguish touching particles with confocal fluorescence microscopy.[12] For quantitative measurements the ability to image separate particles is important, because at the high light intensities used with confocal microscopy the fluorescence of the dye is easily bleached (i.e. the fluorescence is irreversibly destroyed).[12] Therefore, it is dangerous to rely on the fluorescent intensity alone to determine a (local) particle concentration.

The silica particles can be coated with *n*-octadecanol and subsequently dispersed in organic solvents like cyclohexane, chloroform and hexadecane.[13] The refractive index of these solvents lies so close to that of the silica spheres (1.45), that even very concentrated dispersions are hardly turbid. This refractive index matching makes it possible to investigate dispersions with volume fractions of 50% and higher.[12] With latex particles in water, the turbidity usually limits the volume fraction range to lower than 1%.[6, 7]

In hexadecane the octadecanol-coated silica particles form a gel phase if the temperature is lowered below a certain threshold; this gelation is reversible.[12] In Figure 1 the 3-D

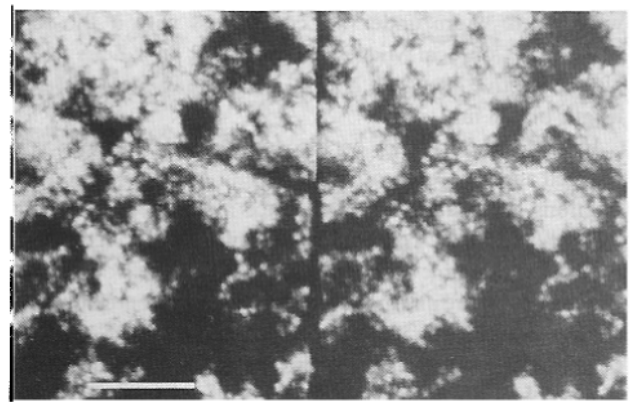

Fig. 1. Stereo pair generated from 10 optical sections with a separation of μm starting 30 μm deep under the cover glass. A thermotropic reversible gel phase of octadecanol-coated silica particles in hexadecane is shown at 20 °C and a particle volume fraction of 15%. The outer radius of the particles is 385 nm (polydispersity in size 2%) and the FITC-labeled fluorescent core has a radius of 204 nm. Bar 10 μm.

structure of such a gel is shown. The particles that were used were labeled with the dye fluorescein-isothiocyanate (FITC) and the optical sections necessary to create the stereo image

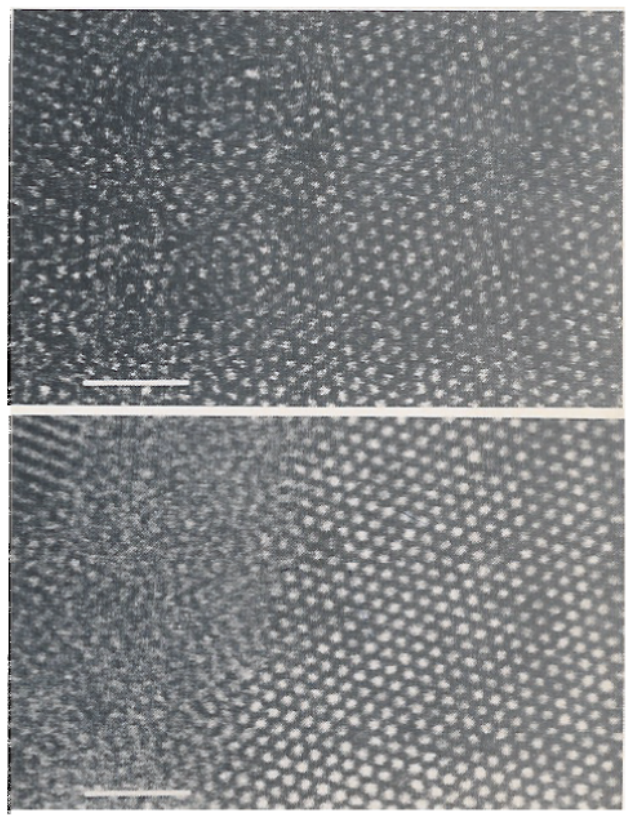

Fig. 2. RITC-labeled octadecanol-coated silica particles in chloroform at a volume fraction of 7% (outer radius 210 nm, fluorescent radius 100 nm, polydispersity 4%). Image taken 30 μm under the cover glass. The inter-particle separation was 0.8 μm because of the charge on the spheres. Bar 5 μm. Top) Averaging over 2 image frames (2 s). Bottom) Averaging over 15 image frames (15 s).

  0935-9648/93/0101-0053 $ 3.50+.25/0

were taken 30 μm under the cover glass with a Bio-Rad MRC-600 Confocal Microscope mounted on a Zeiss Axioplan (objective 40×, oil, N.A. 1.3) and an excitation wavelength of 488 nm.

In chloroform, the octadecanol-coated particles are charged and repel each other.[12] At sufficiently high volume fractions an equilibrium coexistence region between a colloidal liquid and a colloidal crystal phase is formed.[13] In Figure 2 rhodamine-isothiocyanate (RITC) labeled particles in chloroform are imaged at a coexistence concentration (excitation wavelength 568 nm; objective 63×, oil, N.A. 1.4); the closest packed plane of the crystal lay coincidentally parallel with the cover glass. In the ordered solid phase the thermal (Brownian) motion of the spheres is highly localized. In the disordered liquid phase the particles can move over larger distances as is nicely demonstrated by averaging over several frames (compare Fig. 2 top and bottom). The diffuse solid–liquid interface (Fig. 2) is continually fluctuating. In Figure 3 a very thin liquid inclusion layer inside a polycrystalline sample is shown; here the closest packed planes are not parallel to the cover glass.

Again, it is stressed that the images shown in the Figures 2 and 3 are obtained in the *bulk* of the dispersion, far away from wall effects. No equivalent experimental technique exists that will make it possible to obtain similar pictures *inside* a molecular crystal! Therefore, confocal microscopy not only has the possibility to increase our knowledge of concentrated dispersions and materials with structures on the colloidal scale, but may also, if specially prepared colloidal model particles are used, provide us with new information about molecular processes.

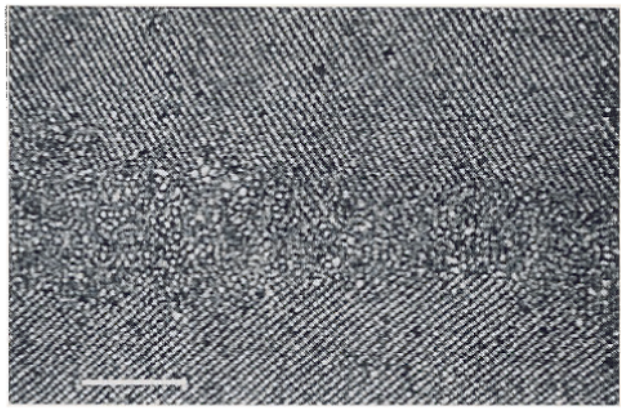

Fig. 3. Same particles and conditions as described for Fig. 2. Thin liquid region enclosed by colloidal crystals.

[1] P. N. Pusey, in *Liquids, Freezing and the Glass Transition* (Eds.: D. Levesque, P.-P. Hansen, J. Zinn-Justin), Elsevier, Amsterdam **1990**.
[2] D. W. Oxtoby, *Nature* **1990**, *347*, 725.
[3] C. G. de Kruif, J. W. Jansen, A. Vrij, in *Physics of Complex and Supermolecular Fluids* (Eds.: S. A. Safran, N. A. Clark), Wiley, New York **1987**, pp. 315–343.
[4] D. J. Klingenberg, C. F. Zukoski IV, *Langmuir* **1990**, *6*, 15.
[5] J. V. Sanders, *Phil. Mag. A* **1980**, *42*, 705.
[6] S. Hachisu, S. Yoshimura, *Nature* **1980**, *283*, 188.
[7] A. Kose, M. Ozaki, K. Takano, Y. Kobayashi, S. Hachisu, *J. Colloid Interface Sci.* **1973**, *44*, 330.
[8] *Confocal Microscopy* (Ed: T. Wilson), Academic Press, London **1990**.
[9] H. Yosida, K. Ito, N. Ise, *Phys. Rev. B* **1991**, *44*, 435.
[10] L. G. B. Bremer, Thesis, University of Wageningen, Wageningen **1992**.
[11] A. van Blaaderen, A. Vrij, *J. Colloid Interface Sci.*, in press.
[12] A. van Blaaderen, A. Imhof, W. Hage, A. Vrij, *Langmuir* **1992**, *8*, 1514.
[13] A. van Blaaderen, A. Vrij, *Langmuir*, in press.

0935-9648/93/0101-0054 $ 3.50+.25/0