Video Article Confocal Imaging of Confined Quiescent and Flowing Colloid-polymer Mixtures

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Abstract

The behavior of confined colloidal suspensions with attractive interparticle interactions is critical to the rational design of materials for directed assembly¹⁻³, drug delivery⁴, improved hydrocarbon recovery⁵⁻⁷, and flowable electrodes for energy storage⁸. Suspensions containing fluorescent colloids and non-adsorbing polymers are appealing model systems, as the ratio of the polymer radius of gyration to the particle radius and concentration of polymer control the range and strength of the interparticle attraction, respectively. By tuning the polymer properties and the volume fraction of the colloids, colloid fluids, fluids of clusters, gels, crystals, and glasses can be obtained⁹. Confocal microscopy, a variant of fluorescence microscopy, allows an optically transparent and fluorescent sample to be imaged with high spatial and temporal resolution in three dimensions. In this technique, a small pinhole or slit blocks the emitted fluorescent light from regions of the sample that are outside the focal volume of the microscope optical system. As a result, only a thin section of the sample in the focal plane is imaged. This technique is particularly well suited to probe the structure and dynamics in dense colloidal suspensions at the single-particle scale: the particles are large enough to be resolved using visible light and diffuse slowly enough to be captured at typical scan speeds of commercial confocal systems¹⁰. In provements in scan speeds and analysis algorithms have also enabled quantitative confocal imaging of flowing suspensions^{11-16,37}. In this paper, we demonstrate confocal microscopy experiments to probe the confined phase behavior and flow properties of colloid-polymer mixtures. We first prepare colloid-polymer mixtures under varying confinement in thin wedge-shaped cells. Finally, we demonstrate a protocol for imaging colloid-polymer mixtures during microchannel flow.

Video Link

The video component of this article can be found at http://www.jove.com/video/51461/

Introduction

This paper demonstrates (a) confocal imaging of quiescent and flowing confined colloid-polymer mixtures in two and three dimensions and (b) particle-tracking and correlation analyses of the resultant images to obtain quantitative information on the phase behavior and flow properties.

Colloidal suspensions with attractive interparticle interactions appear ubiquitously in technological applications as materials for directed assembly¹⁻³, drug delivery⁴, improved hydrocarbon recovery⁵⁻⁷, and energy storage⁸. A common feature of these applications is that the particles must be flowed through fine geometries, such as nozzles, print heads, microchannels, or porous media, and/or be shaped into thin films or rods. Techniques used to probe the structure of micron-sized colloids in confined geometries, including electron microscopy^{17,18}, x-ray microscopy¹⁹, and laser-diffraction microscopy²⁰, can be used to measure the structure and dynamics of particles on the microscale. These techniques, however, do not allow access to the trajectories of individual particles, from which structural and dynamic metrics can be computed for direct comparison to numerical simulations^{21,22}.

Confocal microscopy is a variant of fluorescence microscopy that enables imaging of thin sections of a fluorescent sample. For colloidal science¹⁰, this technique is particularly useful for imaging deep within dense suspensions or in three dimensions. Particle-tracking algorithms²³ applied to two- or three-dimensional time series of confocal micrographs yield the trajectories of all visible particles. As a result, the combination of confocal microscopy and particle-tracking has been applied to study the phase behavior, structure, and dynamics of colloidal suspensions, including ordered crystals²⁴⁻²⁷ and disordered glasses²⁸⁻³¹ and gels³²⁻³⁵.

Other image analysis algorithms can be applied to measure particle dynamics from time series of confocal micrographs. For example, diffusive particle dynamics can be studied by analyzing the fluctuations in intensity over time using confocal differential dynamic microscopy³⁶. When the particle displacements are larger than the interparticle spacing, image correlation³⁷ based on particle image velocimetry³⁸⁻⁴⁰ can be applied to

measure velocity profiles of the particles. The combination of tracking and correlation algorithms has allowed colloidal dynamics to be measured in systems undergoing slow and fast flow^{11-16,41.45}.

We use colloid-polymer mixtures as models for attractive colloidal suspensions⁹. In these mixtures, the range and strength of the attractive interparticle potential are controlled *via* the ratio of the polymer radius of gyration to the particle radius and the concentration of the polymer and the electrostatic repulsion is controlled *via* the addition of a monovalent organic salt⁴⁶. Because the interparticle interactions can be carefully tuned, the solidification of these mixtures has been extensively studied with confocal microscopy ^{34,47-51}.

Here we demonstrate confocal imaging and image analysis³⁷ of quiescent and flowing colloid-polymer mixtures, in which the colloid volume fraction is held fixed at Φ = 0.15, that probe the effect of confinement on the phase behavior and flow properties of these mixtures. These techniques are widely applicable to particulate systems that are refractive index-matched and in which the particles and/or solvent can be labeled with a fluorescent dye.

Protocol

1. Preparation of Colloid-polymer Mixtures

Note: This protocol uses poly(methyl methacrylate) (PMMA) particles, sterically stabilized using poly (12-hydroxystearic acid) and labeled with a fluorescent dye (such as Nile Red, rhodamine B, or fluorescein), that were synthesized following a standard recipe⁵².

- 1. Prepare a 3:1 w/w mixture of cyclohexyl bromide (CXB) and decahydronaphthalene (DHN) as a stock solvent. This mixture nearly matches the density and index of refraction of the particles. Add an organic salt, tetrabutylammonium chloride (TBAC)⁴⁶, to the solvent at a concentration of 1.5 mM to partially screen the charges on the particles.
- 2. To precisely determine the density of the particles, prepare a suspension at approximate particle volume fraction Φ = 0.10 in the CXB:DHN solvent. Centrifuge the suspension at 800 x g for 75 min and add CXB or DHN dropwise to improve the buoyancy matching. In these experiments, the density of the PMMA particles was measured to be ρ = 1.223 g/ml.
- 3. Prepare a concentrated stock suspension of PMMA particles (here, Φ = 0.40) in the CXB:DHN solvent mixture.
- Prepare a concentrated solution of linear polystyrene (PS) in the CXB:DHN solvent mixture. Here, a solution of PS of molecular weight M_w ≈ 3,000,000 (radius of gyration r_q = 15 nm) is prepared at concentration c_p ≈ 50 mg/ml.
- 5. Mix appropriate weights of the particle, polymer, and solvent stock mixtures to formulate suspensions at the desired concentrations of particles and polymers.

Note: Here, suspensions of monodispersed particles are prepared at constant colloid volume fraction $\Phi = 0.15$ and variable polymer concentration in the free volume⁵³ $c_p = 0.25$ mg/ml, and bidispersed suspensions containing two sizes of colloidal particles, with each size bearing a distinct fluorescent label, are prepared at fixed total colloid volume fraction $\Phi = 0.15$, volume fraction ratio of small particles r = 0.50, and polymer concentration in the free volume of 5 or 25 mg/ml.

- 6. After each suspension is prepared, add CXB or DHN dropwise and centrifuge the samples at 800 x g for at least 75 min to confirm that the particles and clusters within the suspension remain buoyancy matched.
- 7. Equilibrate all samples for at least 24 hr prior to imaging experiments.

2. Quiescent Sample Experiments: Phase Behavior

- 1. To determine the bulk phase behavior, fabricate rectangular chambers from glass coverslips (**Figure 1a**). For the colloid-polymer mixtures in this study, chambers of thickness *h* = 1 mm (set by the thickness of a microscope slide) give bulk behavior.
- To access multiple confinements in a single microscopy experiment, fabricate thin wedge-shaped chambers, using a single coverslip as a spacer on one wedge (Figure 1b). The opening angle of the chamber is <0.5°, so that in a single field of view the walls are very nearly parallel. A representative chamber allows access to confinement thicknesses of h = 6 to >100 µm.
- 3. Build chambers on a coverslip base for imaging on an inverted microscope and seal with UV-curable epoxy, which does not dissolve in the CXB-DHN solvent mixture.
- 4. Image samples using a confocal microscope. This protocol demonstrates imaging with a line-scanning confocal attached to an inverted microscope equipped with a 100X oil immersion lens of numerical aperture NA = 1.40.
- 5. Excite the dyes using a laser source. Here wavelengths λ = 491 or 561 nm are used to excite the fluorescein and rhodamine/Nile Red dyes, respectively.
- 6. In the point-scanning system, generate an image by rapidly scanning the focal point across the sample (in the x-y plane) using the confocal software. A two-dimensional image of 512 pixels x 512 pixels, covering approximately 50 µm x 50 µm, can be acquired in 1/32 sec. Improve the image quality by averaging multiple images or increasing the acquisition time.
- Locate the bottom of the chamber (z = 0), for example by focusing on particles adhered to its bottom. In this setup, the height (z) increases with increasing focus into the chamber.
- As an example, characterize the effect of confinement on the dynamics of the particles by acquiring a 2-D time series of images (in the x-y plane) at the midplane of the chamber. In a typical experiment, 500 images of dimension 512 pixels x 512 pixels are acquired at a frame rate of 1 frame/sec (time spacing ∆t = 1 sec).
- As a second example, characterize the 3-D structure of particles by acquiring a three-dimensional series of images (x,y,z). In a typical experiment, two-dimensional images (512 pixels x 512 pixels) are acquired at multiple z positions within the chamber, with a constant spacing of Δz = 0.2 µm between consecutive images set by a piezo. A volume stack covering a thickness of h = 30 µm thus contains 151 images.
 Locate and track particles over time in 2-D or 3-D using particle-tracking software written in IDL^{23,54-56}, MATLAB^{57,58}, LabView⁵⁹, or Python⁶⁰.
- 10. Locate and track particles over time in 2-D or 3-D using particle-tracking software written in IDL^{23,54-56}, MATLAB^{57,58}, LabView⁵⁹, or Python⁶⁰. These algorithms typically allow the centers of the particles to be resolved within 40–50 nm. Successful particle tracking requires that the particles move less than the interparticle spacing between consecutive frames.

11. From the particle positions, calculate structural and dynamic metrics. Three convenient metrics shown here are the 3-D pair correlation function $g(r)^{61}$, the 2-D mean-squared displacement (MSD)^{58,62}, and the 2-D self part of the van Hove correlation function $G_s(x,t)^{58}$. The latter two metrics can be also calculated in 3-D.

3. Flowing Experiments: Flow Properties

- 1. Fabricate a simple flow cell using a glass microcapillary with square cross-section (100 µm x 100 µm) that is affixed to Teflon tubing. Use glass coverslips to support the capillary and provide mechanical rigidity, as shown in the schematic in Figure 7. 2.
 - Load the colloid-polymer mixture into a glass syringe. Attach the syringe to a syringe pump or a pneumatic fluid dispensing system.
- Mount the flow cell setup onto the inverted microscope. Keep the syringe, flow cell, and outlet at the same height to minimize the effect of 3 gravity on the flow profile.
- Control the flow rate of the suspension through the flow cell by the volumetric flow rate (for the syringe pump) or the applied pressure (for the 4. pressure box). The average velocity of the suspension in the microchannel also depends upon the suspension formulation. Typical values of the maximum velocity in the square microchannel measured here are 200-2,000 µm/sec.
- 5. During flow, acquire a 2-D confocal time series at fast frame rates. Here, 500 images of dimension 512 pixels x 512 pixels are acquired at 32 frames/sec (time spacing Δt = 1/32 sec) at different heights above the bottom of the microchannel (z = 0 µm) ranging from z = 5–50 µm. Each image covers roughly half of the lateral dimension (y) of the microchannel, as shown in the inset to Figure 7. If the particles appear elliptical, increase the frame rate of acquisition.
- 6. As in quiescent experiments, locate the particles in 2-D using standard algorithms for locating and tracking particles in IDL or MATLAB. For slow flows, in which particles move less than the average interparticle distance between frames, use tracking algorithms to obtain the trajectories.
- Use image correlation to calculate the velocity profiles for fast flows. 7.
 - 1. Subdivide the image into horizontal images of constant height (y) along the direction of flow (x). For two sequential images $I_1(x,y)$ and $I_2(x,y)$ shift the latter image by a factor Δx and then calculate the cross-covariance between $I_1(x,y)$ and $I_2(x+\Delta x,y)$.
 - 2. Identify the peak position of the histogram of Δx values that maximize the cross-covariance between each pair of images to obtain the mean advection velocity at each lateral position y. If this distribution is not strongly peaked, acquire images at a faster frame rate.

Representative Results

To demonstrate confocal imaging and particle-tracking, we investigated the effect of confinement on the phase behavior of colloid-polymer mixtures⁶³⁻⁶⁵. For these experiments the colloid diameter was $2a = 0.865 \mu m$. The colloid volume fraction was fixed at $\Phi = 0.15$ and the concentration of polymer c_p was varied from 0 to 23.6 mg/ml. Representative confocal images are shown in Figure 2⁶³, left column. From the particle positions obtained using tracking algorithms, we calculated representative structural and dynamic metrics, including the pair correlation function (Figure 2, center column) and the mean-squared displacement (Figure 2, right column). The images and the structural and dynamic metrics allowed us to distinguish between colloidal gels (Figure 2, top row), fluids of clusters (Figure 2, middle row), and fluids (Figure 2, bottom row)

These metrics allowed us to quantitatively assess confinement-induced changes in particle structure and dynamics. For example, the first peak in the pair correlation function of a sample with polymer concentration $c_p = 16.6$ mg/ml moves to shorter separations as the confinement thickness is decreased (**Figure 3⁶³**). Similarly, the self part of the van Hove correlation function for a sample with c_n = 16.6 mg/ml narrows as the confinement thickness is decreased and at the smallest confinement exhibits the highly non-Gaussian profile of an arrested gel (**Figure 4**⁶³). Even for a sample with c_{o} = 23.6 mg/ml, which is an arrested gel at all confinements, decreasing the confinement thickness leads to a narrower and more non-Gaussian profile. These changes suggested that confinement indirectly induced an increase in the effective interparticle attraction.

These techniques could also be applied to assess the effects of confinement in bidispersed mixtures of particles bearing distinct fluorescent labels. Representative images of a mixture of large particles (diameter $2a_L=1.48 \mu m$) and small (diameter $2a_S = 0.73 \mu m$) particles, with a total particle volume fraction $\Phi_{tot} = 0.15$ and a volume fraction ratio of small particles r = 0.50, show that decreasing the confinement thickness induces the formation of a solid-like colloidal gel phase containing both large and small particles (Figure 5⁸⁵). This effect is especially pronounced for suspensions that are bulk fluids (Figure 5a-d). The MSDs of the large particles decrease as the system is increasingly confined: for a sample with $c_n = 5$ mg/ml, the MSDs become dynamically arrested, consistent with the solidification transition (**Figure 6a**⁶⁵). For a sample with c_p = 25 mg/ml, which forms an arrested gel, strong confinement induces slowed dynamics (Figure 6b); the differences between the mostconfined and least-confined sample are larger than the resolution of this technique (~40 nm, which we determine from the y-intercept of the MSD in a very dilute concentration of particles with $\Phi < 0.01$).

Confocal microscopy and image analysis also enable differences between the flow properties of weakly and strongly attractive particles during confined flow to be characterized⁶⁶. For these experiments, suspensions of particles of diameter 2a = 1.55 µm were concentrated to a volume fraction of $\Phi = 0.15$ with a polymer concentration of either $c_p = 5$ mg/ml, corresponding to a weak interparticle attraction, or $c_p = 25$ mg/ml, corresponding to a strong interparticle attraction. Representative images are shown in Figure 8 for quiescent and flowing suspensions.

Number density profiles revealed differences between the distribution of particles in weakly- and strongly-attractive suspensions during flow (shown for a flow rate of 10 μ l/hr in Figure 9⁶⁶): those of a weakly-attractive suspension ($c_p = 5 \text{ mg/ml}$, Figures 9a and 9b) increase more dramatically towards the center of the microchannel than those of a strongly-attractive suspension ($c_n = 5$ mg/ml, Figures 9c and 9d).

Similarly, weakly and strongly attractive suspension exhibited differences in the shape of velocity profiles (shown for a flow rate of 8 µl/hr in Figure 10⁶⁶). The velocity profiles for suspensions with a weak attraction ($c_p = 5 \text{ mg/ml}$, Figures 10a and 10b) are close to those predicted for a Newtonian fluid flowing in a square microchannel. By contrast, the velocity profiles for suspensions with a stronger attraction ($c_n = 25 \text{ mg/ml}$, Figures 10c and 10d) deviate significantly from the Newtonian profile near the lateral walls of the microchannel (near y/L = 0).





Figure 1. Schematic of chambers for quiescent experiments. (a) Rectangular chamber, with a constant thickness of 1 mm set by the thickness of a glass slide. (b) Wedge-shaped chamber, with a variable thickness ranging from 6-170 μ m. The maximum thickness is set by that of a glass coverslip.



Figure 2. Distinguishing features of fluids, fluids of clusters, and gels. Left column: Confocal micrographs. Center column: Particle pair correlation function g(r) as a function of the normalized separation r/2a. Right column: Normalized mean-squared displacement MSD/4 a^2 as a function of lag time. From top to bottom the images and correlation functions show representative examples of a gel (c_p = 23.6 mg/ml and h/2a = 8.7), a fluid of clusters (c_p = 16.6 mg/ml and h/2a = 17), and a colloidal fluid (c_p = 0 mg/ml and h/2a > 116). The particle diameter is 2a = 0.865 μ m. This figure has been modified from Spannuth and Conrad, *Phys. Rev. Lett.* **109**, 028301 (2012). Copyright 2012 by the American Physical Society.



Figure 3. Pair correlation function indicates structural changes in confinement. Pair correlation function for a sample with c_p = 16.6 mg/ ml at h/2a > 116 and h/2a = 69, 35, 17, and 8.7 from top to bottom. Sample is a fluid of clusters at all *h* except h/2a = 8.7, which is a gel. Colors indicate the phase as in **Figure 2**. The particle diameter is $2a = 0.865 \mu$ m. This figure has been modified from Spannuth and Conrad, *Phys. Rev. Lett.* **109**, 028301 (2012). Copyright 2012 by the American Physical Society.





Figure 4. van Hove correlation function and the mean-squared displacement indicate changes in confinement. Self part of the van Hove correlation function and (inset) mean-squared displacement for samples with (**a**) $c_p = 16.6$ mg/ml and (**b**) $c_p = 23.6$ mg/ml as a function of confinement (h/2a = 69 (circles), 17 (squares), and 8.7 (triangles)). In (**a**), the sample is a fluid of clusters for h/2a = 69 and 17, but a gel for h/2a = 8.7. In (**b**), the sample is a gel for all *h*. The particle diameter is $2a = 0.865 \ \mu$ m. This figure has been modified from Spannuth and Conrad, *Phys. Rev. Lett.* **109**, 028301 (2012). Copyright 2012 by the American Physical Society.



Figure 5. Representative confocal micrographs of bidispersed mixtures. Confocal micrographs of small and large particle populations (both shown in gray, with larger particles having greater intensity), for suspensions with a constant total volume fraction $\Phi_{tot} = 0.15$, volume percent of small particles *r* = 0.50, concentration of depletant polymer c_p (**a-c**) 5 mg/ml or (**d-f**) 25 mg/ml, (**a,d**) in bulk or (**b-c**, **e-f**) confined to a normalized height $h/2a_L$ of (**b,e**) 20, or (**c,f**) < 5.5. The large and small particle diameters are $2a_L = 1.48 \ \mu m$ and $2a_S = 0.73 \ \mu m$, respectively. The scale bar is 10 μm .



Figure 6. Mean-squared displacement of large particle population exhibits confinement-induced slowing. Normalized mean-squared displacement as a function of lag time *r* of large particles in binary suspensions with volume percent of small particles *r* = 0.50 and varying concentration of depletant polymer of (**a**) $c_p = 5$ mg/ml or (**b**) $c_p = 25$ mg/ml. Confinement thicknesses $h/2a_L$: bulk (\Rightarrow), 41 (Δ), 20 (\diamond), and <5.5 (\triangleleft). The dashed lines indicate a slope of 1. The large particle diameter is $2a_L = 1.48 \ \mu$ m. This figure has been modified from Pandey and Conrad, *Soft Matter*, **9**, 10617-10626 (2013). Reproduced by permission of the Royal Society of Chemistry (http://dx.doi.org/10.1039/c c3sm51879e).



Figure 7. Schematic of device for microchannel flow experiments. The device consists of a glass microcapillary that is attached to a syringe pump using Teflon tubing. The schematic as shown is inverted compared to the experimental setup, which uses an inverted microscope. The coordinate system is indicated: x is oriented along the direction of flow, y is oriented along the width of the microchannel, and z is oriented along the vertical direction. Inset: schematic of imaging protocol. 2-d movies are acquired of half the width of the microchannel at ten different heights above its bottom surface. This figure has been modified from Pandey and Conrad, *Soft Matter* **8**, 10695-10703 (2012). Reproduced by permission of the Royal Society of Chemistry (http://dx.doi.org/10.1039/c2sm25935d).



Figure 8. Representative confocal micrographs of particles during microchannel flow. Confocal micrographs of colloidal suspensions with polymer concentration c_p of (**a-c**) 5 mg/ml and (**d-f**) 25 mg/ml. Images (**a**) and (**d**) show the quiescent sample; images (**b-c**) and (**e-f**) were acquired during flow at a height (z) of (**b,e**) 5 μ m and (**c,f**) 50 μ m above the bottom surface of the microchannel. The edge of the channel is at the left-hand side of each image in (**b-c**) and (**e-f**), and flow is in the downward direction as indicated. The scale bar is 10 μ m. The particle diameter is 2*a* = 1.546 μ m. This figure has been modified from Pandey and Conrad, *Soft Matter* **8**, 10695-10703 (2012). Reproduced by permission of the Royal Society of Chemistry (http://dx.doi.org/10.1039/c2sm25935d).



Figure 9. Strength of attraction modifies the number density profile of particles. Number of particles as a function of normalized lateral position across the microchannel y/L for suspensions with volume fraction $\Phi = 0.15$ and polymer concentration c_p of (\mathbf{a}, \mathbf{b}) 5 mg/ml and (\mathbf{c}, \mathbf{d}) 25 mg/ml, acquired at normalized downstream positions $x/L = (\mathbf{a}, \mathbf{c})$ 50 and (\mathbf{b}, \mathbf{d}) 200. Data are shown for two normalized heights, z/L = 0.05 (dark red Δ) and 0.50 (light blue \triangleright). The flow rate was 10 μ l/hr. The lines shown are guides to the eye, and the error bars indicate the standard deviation of the measurements. The particle diameter is $2a = 1.546 \mu$ m. This figure has been modified from Pandey and Conrad, *Soft Matter* 8, 10695-10703 (2012). Reproduced by permission of the Royal Society of Chemistry (http://dx.doi.org/10.1039/c2sm25935d).



Figure 10. Strength of attraction modifies the velocity profiles. Normalized velocity profiles as a function of normalized lateral position across the microchannel y/L for suspensions with volume fraction $\Phi = 0.15$ and polymer concentration c_p of (**a**,**b**) 5 mg/ml and (**c**,**d**) 25 mg/ml, acquired at normalized downstream positions $x/L = (\mathbf{a}, \mathbf{c})$ 50 and (**b**,**d**) 200. Data are shown for two normalized heights, z/L = 0.05 (dark red) and 0.50 (light blue). The flow rate was 8 μ l/hr. The error bars indicate the standard deviation of the measurements. The dotted lines indicate fits to the Newtonian flow profile expected for flow in a square microchannel. This figure has been modified from Pandey and Conrad, Soft Matter 8, 10695-10703 (2012). Reproduced by permission of the Royal Society of Chemistry (http://dx.doi.org/10.1039/c2sm25935d).

Discussion

Colloidal suspensions are widely studied as models for confined phase behavior, because micron-sized colloidal particles exhibit significantly slower dynamics than atoms and molecules and thus can be readily imaged and tracked over time¹⁰. For these fundamental studies, understanding the effect of interparticle attractions on confined phase behavior offers the opportunity to explore phenomena such as capillary condensation and evaporation^{21,22,67}. In addition, confined attractive suspensions appear ubiquitously in industrial and technological applications. For these applied studies, understanding the effect of suspension formulation on confined flow may lead to improved formulations of feedstocks and inks for rapid prototyping¹⁻³, drug delivery⁴, enhanced oil recovery⁵⁻⁷, and energy storage applications⁸.

Other techniques used to probe the structure of micron-sized colloids in thin geometries include electron microscopy^{17,18}, x-ray microscopy¹⁹, and laser-diffraction microscopy²⁰. The combination of confocal microscopy and image analysis algorithms offers two key advantages for studies of confined colloidal suspensions. First, confocal microscopy allows imaging deep within dense and fluorescent suspensions. Second,

particle trajectories obtained from the confocal micrographs can be used to calculate dynamic and structural metrics for individual micron-sized colloidal particles. These measurements facilitate direct comparison with simulations^{21,22}. Other image analysis algorithms, such as image correlation³⁷ based on particle image velocimetry³⁸, can be applied to confocal micrographs in which the particle displacements are larger than the interparticle spacing to probe the dynamics.

We have demonstrated protocols for imaging quiescent and flowing colloid-polymer mixtures in confined geometries using confocal microscopy. Using the representative metrics outlined in this paper, we identified a solidification transition in quiescent suspensions that is induced by confinement^{63,64}. We also showed that changing the strength of attraction modifies the extent of particle migration and consolidation during flow of colloid-polymer mixtures in microchannels⁶⁶.

For flow experiments, one limitation of this protocol is the difficulty in imaging in 3-D during fast microchannel flow, as particles typically move large distances between consecutive frames. This limitation can be avoided at slower flow rates by imaging a thin 3-D slice. Repeated experiments at distinct vertical positions within the channel can thus yield the full three-dimensional velocity and density profiles. This approach has been successfully applied by other research groups to suspensions of nearly-hard-sphere colloids that form glasses^{13,14} or crystals⁴¹ but have not yet been applied to the less concentrated colloidal gels such as those presented here.

Both image analysis methods described (particle-tracking and image correlation) exhibit limitations. For particle tracking algorithms, resolutions of one-tenth of the pixel size have been reported²³ for well-separated particles. The resolution increases with increased signal-to-noise. Both the Gaussian fitting algorithm and a newer algorithm that calculates the points of maximal radial symmetry achieve tracking resolutions that are close to the theoretical maximum⁶⁸. The resolution in our experiments was somewhat less (one-fourth of the pixel size); because the particles in gels are in close contact, the center of intensity in a local region is thus not necessarily located at the particle centroid. The resolution in the tracking of particles in gels can be improved by labeling only the cores of the particles⁶⁹⁻⁷¹. For particle image velocimetry, the methods reported here yield accurate measurements of displacement even for large displacements of several hundred pixels³⁷, so long as regions of near-constant advective velocity are correlated. These methods cannot be used when particles undergo significant relative motion or non-affine motion; in this case displacements are limited to a fraction of the particle size.

These protocols can be applied to studies of the phase behavior, structure, dynamics, and flow properties of a wide variety of colloidal suspensions, including particles with shape or chemical anisotropy and solvents that are non-Newtonian fluids, in 2-D and 3-D confinements. Applying these techniques requires further increase in the imaging rate in 2-D and 3-D, new algorithms to analyze images of anisotropic particles, and advances in fabrication of realistic confining geometries and interfaces.

Disclosures

The authors have nothing to disclose.

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