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Confocal Microscopy of Colloidal Suspensions

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Colloidal systems or colloids consist of microparticles or nanoparticles (solute) uniformly suspended in a liquid (solvent), also called colloidal suspensions. They can mimic and exhibit microscopic or atomic aspects of molecular and atomic systems. They have been increasingly studied because of their similarity with atomic systems. They can be microscopically observed by optical microscopes because they are large enough in size and slow in motion to be monitored; microscopic methods are very useful and powerful in research on colloidal systems. Recently, confocal laser microscopy has been known as a powerful tool to obtain information of real-space and real-time behaviors of colloidal suspensions. In particular, it is possible to exactly track individual colloids in three dimensions with confocal microscopy. In this article, we briefly discuss the usefulness of confocal microscopy in colloidal systems that are currently used as model systems to resolve important questions in materials science.

Key Words: Colloidal systems, Colloids, Colloidal suspensions, Confocal microscopy

INTRODUCTION

The study of the microstructures, growth dynamics, and physical properties of crystals is one of the most important topics of condensed matter physics and materials science (Palberg, 1999). Although many behaviors of bulk crystals are already known well, quite less is known about their earliest stages when they undergo nucleation and growth from liquid (Gasser et al., 2001). The main reason of difficulties in investigation of the earliest stages is that atoms in real systems are too small in size and much fast in motion; therefore direct observations of the dynamic atomic behaviors and processes are not available so far with conventional visualization techniques. To overcome these difficulties, colloidal model systems and confocal microscopic observations can be alternatives. With this approach, we are able to directly observe three-dimensional microstructures and to experimentally explore the physical signatures of atomic systems with colloidal systems. Colloidal particles are small enough to display the Brownian motion at room temperature as well as their interactions are easily modified to imitate atomic interactions. Thanks to the thermodynamic analogy, it is then possible to perform studies related to atomic systems through the use of colloidal systems (Zhang & Liu, 2014). Additionally the individual particle tracking leads to the detailed dynamics of individual colloids in real situations. These features from in-situ confocal imaging of colloidal particles are helpful in our understanding of static and dynamic phenomena of colloidal model systems.

In this article, we briefly summarize recent advances in confocal imaging of colloidal model systems. Direct visualization of colloidal model systems with confocal microscopy enables us to elucidate the real dynamics of colloidal suspensions. We describe an example for colloidal model systems that can be studied with confocal microscopy.

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COLLOIDAL MODEL SYSTEMS

Colloidal model systems were developed three decades ago in order to investigate the phenomena of nanoparticles in suspensions, such as the crystal growth dynamics and the phase transitions (Murray et al., 1996). The term 'colloid' describes a multiphasic substance that is made of particles, roughly from 10 nm to 10 μm in diameter, uniformly dispersed in a continuous phase (i.e., liquid). The dispersed particles in a solution usually exhibit the Brownian motion. Colloidal particles are small enough that thermal fluctuations are definitely significant in the solution (Habdas & Weeks, 2002). On the other hand, colloidal particles can show equilibrium phase transitions among solid, liquid, and gas, which are similar to phase transitions in actual atomic systems (Anderson & Lekkerkerker, 2002). Therefore, each colloidal particle can be regarded as an atom despite different scales.

Colloidal suspensions are powerful model systems for investigating the phase transitions that are alike in actual atomic systems. There are many advantages in using colloidal model systems as follows. The first advantage comes from the particle size, which is large enough for real-space observations with optical microscopy, and their relatively slow motion, which is tractable in real time at the single-particle level. The second advantage comes from the versatile modifications of colloidal particles, which are useful to make various colloidal systems. An important feature of colloidal systems is that the interactions between colloidal particles can be easily modified to be from attractive to repulsive, from short-range to long-range, from hard to soft, and from symmetric to directional (Zhang & Liu, 2014). Consequently, colloidal model systems are visible with optical microscopy and useful in studying on atomic-like behaviors by tuning particle sizes and material properties of colloids.

CONFOCAL MICROSCOPY OF COLLOIDS

A confocal laser microscope is a laser scanned optical microscope that utilizes a fluorescent technique (Olafsen, 2010). The fluorescent dye included in a sample is excited by the laser light, emitting fluorescence through a sequence process (Olafsen, 2010). Distinct from conventional fluorescence microscopies, confocal microscopy consists of a laser source, pinhole, and detector; particularly the detector is much more sensitive than human eyes. A schematic illustration of confocal microscopy is shown in Fig. 1. Here, laser light (marked by the yellow line) is reflected by a dichroic mirror to a rotating mirror which scans the light in x- and y-axes. The light then goes through the microscope and excites the fluorescent dye from the sample. The fluoresced light (marked by the orange line) from the fluorescent sample goes back through the microscope and is descanned by the same mirror (Prasad et al., 2007). The light then passes through the dichroic mirror without reflection and reaches a screen through a pinhole, which is placed in the conjugate focal plane of the sample. The pinhole removes the out-of-focused light arriving from the sample and guides the in-focused light to the detector. By scanning laser on the sample, magnified images of the focused area are attainable.

The image acquisition time is very rapid, so that we can take

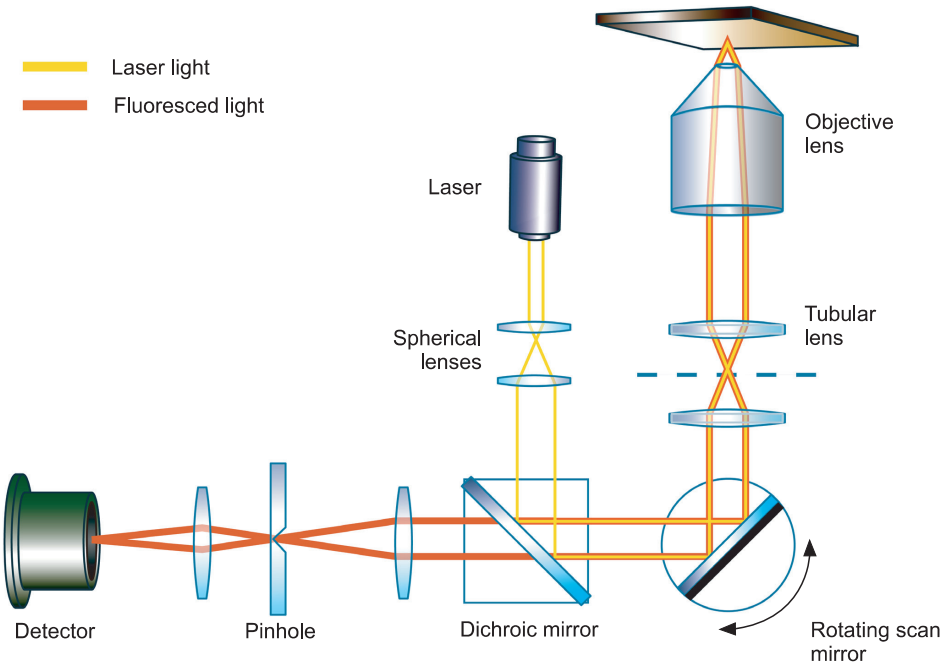

Fig. 1. A schematic illustration of confocal microscopy that usually consists of a laser source, microscope (a series of lenses), detector, and pinhole. Fluorescent dyes are used to track individual molecules.

a dynamic motion of colloids in a suspension. Fig. 2 shows an example of colloidal particles that move on a drying droplet (Weon & Je, 2010). In particular, these sequential images (Fig. 2A) show that the evaporation processes such as pinning, packing, and percolation are distinguishable owing to different fluorescence intensities among air (black), solvent (dark green), and colloids (bright green) (Fig. 2B).

At a focal plane (z-axis), the two-dimensional images are collected by computer. The two-dimensional slices taken at different depths (focal planes) are combined and reconstructed as a three-dimensional image. Fig. 3 shows an example for three-dimensional imaging of a double emulsion sample. The three-dimensional reconstruction can dramatically expand the applications of confocal microscopy.

Another important feature of confocal microscopy is the optical section of the sample, which enables us to see the internal structure without damages by the confocal mode to get clear image without blurring. It is also useful in characterizing three-dimensional internal structures without any destructions of the sample as seen in Fig. 3. From the three-dimensional imaging, it is possible to track the particle position where the fluorescence dyed particles are placed in a solution. In case of thick samples, high-resolution imaging can be achieved with confocal microscopy.

Many progresses have been made by adopting confocal microscopy in understanding of colloidal systems thanks to advances in imaging and particle tracking. The early studies of colloidal systems with confocal microscopy have been extensively conducted in condensed matter physics and materials science (Pusey & van Megen, 1986). One of the early studies of colloids using a confocal microscope was related to the influence of a glass-suspension interface on colloidal crystals (Yoshida et al., 1991). The applications of confocal microscopy have been extended to explore the internal dynamics of the dense colloidal systems that consist of microspheres with packing fractions of ~60% (van Blaaderen & Wiltzius, 1995). Recent advances in image analysis algorithms were essential to study high-density suspensions. Micrometer-sized colloidal systems are now visualized clearly and easily by confocal microscopy.

CRYSTAL NUCLEATION

Nucleation is the kinetic process that relates the formation of a nucleus from a liquid state. This is a critical process that initially occurs in crystallization. By nucleation, the early embryos are dynamically generated from supersaturated liquid clusters to grow up as crystals. To make large crystals with better qualities, it is necessary to elaborately control the dynamic nucleation process. A complete understanding of nucleation is required in the elaborate nucleation control. The most widely known theory about nucleation is the classic nucleation theory that provides a nice framework for understanding crystallization. According to the classical description, the crystallization kinetics goes on two part, nucleation and growth of nuclei. However, previous studies have found different results from the classical theory (Mostafa & Richard, 1982). Some people discovered that the classical theory works merely in specific conditions. For this reason,

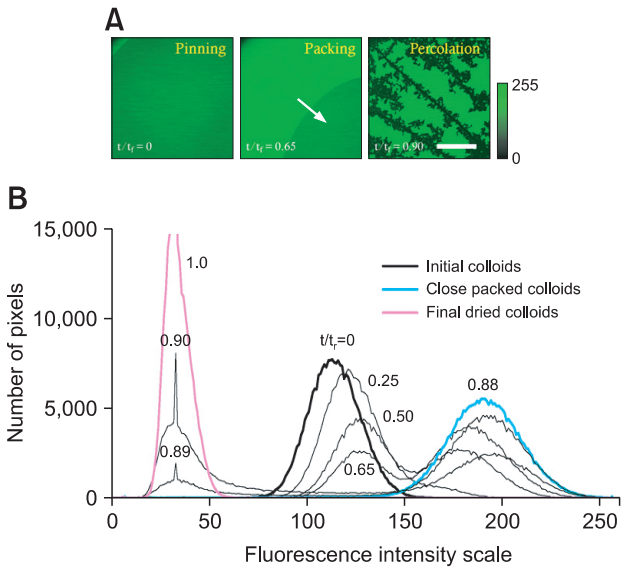

Fig. 2. An example of direct visualization of colloidal particles that move on a drying droplet. The evaporation processes such as pinning, packing, and percolation are distinguishable (A) owing to different fluorescence intensities among air (black in A), solvent (dark green in A), and colloids (bright green in A) (B).

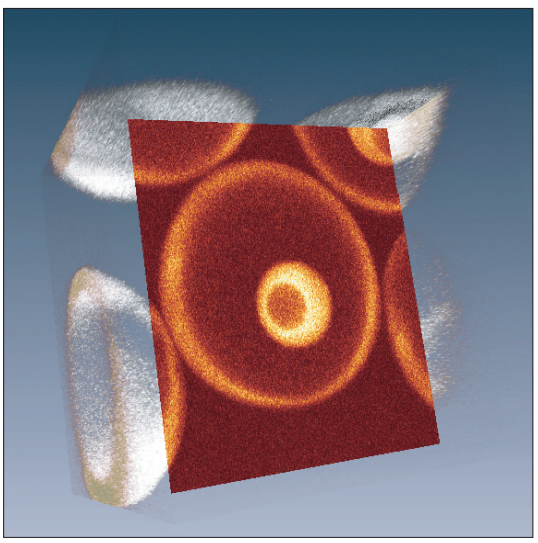

Fig. 3. An example for three-dimensional reconstruction of a double emulsion sample. This shows a non-destructive visualization of the internal structures.

there exist some discrepancies between the result from the experiment and the classical theory (Fokin & Zanotto, 2000). These discrepancies suggest that the classical theory must be limited and there is a possibility of a new nucleation theory. Colloidal model systems using confocal microscopy can be a solution of this topic. Colloidal systems provide direct experimental evidence for the crystallization process, whereas computer simulations, for instance, Monte Carlo simulations offer indirect information (ten Wolde et al., 1995; Battaile, 2008). As an important example, most recently a direct visualization of colloidal systems with confocal microscopy was used to identify that there are dynamic kinetic pathways in nucleation processes, which are different from the classical nucleation theory (Tan et al., 2014). The formation of crystal nuclei is mediated by a transient intermediate ordered precursor phase during liquid-to-solid phase transitions (Tan et al., 2014). This finding is in contrast to the classical assumptions of that the structure of nuclei would be identical to the structure of the bulk phase. Further studies about the intermediate pathways of nucleation are required in terms of more observations and physical mechanisms. We believe that direct experimental visualizations of colloidal model systems with confocal microscopy will be essential in this topic in basic physics and materials science.

CONCLUSIONS

In conclusion, here we described the usefulness of confocal microscopy in colloidal systems that are currently used as model systems to study about the important questions in materials science, for instance, the nucleation kinetics. Colloidal systems can show behaviors like actual atomic systems despite different scales, and many unknown phenomena regarding colloidal systems can be resolved in real space and real time by confocal microscopy. Confocal microscopy is appropriate for direct observations of colloidal systems due to powerful features of individual particle tracking and three-dimensional reconstruction. We believe that confocal microscopy of colloidal model systems would be greatly useful to uncover many mysteries in materials science.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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