Phase transitions and interfaces in temperature-sensitive colloidal systems
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Chapter 2

Experimental Techniques

In this thesis, we investigate colloidal phase behavior and the interfaces at the particle level in both real space and reciprocal space. The particle sizes are in the range of several hundred nm to \( \mu \)m, therefore, the standard experimental methods used in this thesis are light scattering and confocal microscopy. Below we describe in detail the basic principle of each techniques and the information obtained by them. We also show the standard methods of sample preparation, particle size measurement and temperature control techniques.

2.1 Dynamic light scattering

When colloidal particles are suspended in a solvent, the particles exhibit a random motion called Brownian motion. Each particle exhibits a zigzag motion, and its motion is uncorrelated with the other particles in very dilute suspensions. As light scatters from the moving particles, this motion imparts a randomness to the phase of the scattered light, such that when the scattered light from two or more particles is added together, there will be a changing destructive or constructive interference. This gives rise to time-dependent
fluctuations in the intensity of the scattered light, known as “speckle patterns”. The fluctuations are quantified by the intensity autocorrelation function [1, 2]

$$\langle I(q,t)I(q,0) \rangle = \lim_{T \to \infty} \frac{1}{T} \int_{0}^{T} I(q,\tau)I(q,\tau+t)d\tau$$

(2.1)

where \(q\) is the scattering wave-vector, \(I(q,0)\) and \(I(q,t)\) are the scattered intensity at time 0 and time \(t\), respectively. The brackets denote an ensemble average. The scattering wave-vector is directly related to the laser wave length \(\lambda\) and scattering angle \(\theta\) as

$$q = (4\pi/\lambda)\sin(\theta/2)$$

(2.2)

A sketch of the light scattering setup is shown in Fig. 2.1.

In Dynamic Light Scattering (DLS) measurements, the time fluctuations of the scattered intensity are sent into a correlator that calculates the normalized intensity correlation function in real time as

$$g^1(q,t) = \frac{\langle I(q,0)I(q,t) \rangle}{\langle I(q) \rangle^2}$$

(2.3)

where \(\langle I(q) \rangle\) is the ensemble averaged scattered intensity. For simple diffusion of the particles, this correlation function decays exponentially with time as

$$g^1(q,t) = \exp(-2Dq^2t)$$

(2.4)

where \(D\) is the diffusion coefficient. The hydrodynamic radius \(R\) of the particles is determined directly from the diffusion coefficient using the Einstein-Stokes relation:
\[ R = \frac{k_B T}{6\pi\eta D} \]  

where \( \eta \) is the viscosity of the solvent, \( k_B \) is Boltzman’s constant, and \( T \) is temperature of the solvent.

### 2.2 Confocal microscopy

#### 2.2.1 Confocal microscopy setup

The Laser Scanning Confocal Microscope (LSCM) was invented in 1957 by Marvin Minsky [3]. The confocal microscope uses point illumination and a pinhole in an optically conjugate plane in front of the detector to eliminate out-of-focus light. Only the light within the focal plane is detected, so the LSCM provides 2D sections though a 3D specimen. As only one point is illuminated at a time in LSCM, 2D or 3D imaging requires scanning over a regular raster (i.e. a rectangular pattern of parallel scanning lines) in the specimen. The thickness of the focal plane is defined mostly by the point spread function due to the diffraction through the circular aperture (pinhole).

![Schematic configuration of the confocal microscope setup.](image)

**Figure 2.2**: Schematic configuration of the confocal microscope setup.
A LSCM shows much better contrast than a conventional microscope because the detector pinhole blocks most scattered and out of focus light. Also, scattered light is reduced because only a small part of the sample is illuminated. Fluorescent samples give good contrast because a filter can block out everything except the fluorescent wavelengths. With a dichroic mirror, a single objective can be used so that the laser and the fluorescent light go through the same objective (Fig. 2.2). A conventional LSCM can take an image in about one second. Here, we use a recent fast Zeiss LSM 5 Live confocal microscope that can acquire up to 120 frames per second at a resolution of 512 by 512 pixels.

2.2.2 Data acquisition

The most widely used particle tracking algorithm was introduced by Crocker and Grier in 1995 [4]. By assuming the particles appear as spherical bright spots on a dark background, the software defines local brightness maxima within an image as candidate particle locations. Then the tracking algorithms remove the undesired noise and long wavelength contrast gradients using a spatial band pass filter. A centroid algorithm finds the center of particles by locating the brightness weighted center of mass of the bright spots. With this refinement procedure the coordinates of the particle centers can be obtained with sub-pixel resolution down to less than 1 / 10 of the pixel size. We show an example of the different stages of the particle tracking procedure in Fig. 2.3; the confocal image (a), the image after using the spatial band pass filters (b), and the determined particle centers, shown as the black dots within the white spots (c).

Figure 2.3 : Particle tracking procedure:

(a) A confocal image. (b) The image after using spatial band pass filter. (c) The particle centers are shown as the black dots within the white spots.
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One of the most advanced features of the software is linking particle locations into trajectories. The program matches up locations in each image with corresponding locations in later images to produce particle trajectories. This requires determining which particle in a given image most likely corresponds to one in the preceding image. Linking particle positions into trajectories is only feasible if the typical single particle displacement between subsequent images is sufficiently smaller than the typical inter-particle spacing, $a$. Otherwise, particle positions will become inextricably confused between snapshots. The optimal cutoff parameter falls in the range of $\delta \leq a/2$. Any particle with no match in the successive frames is considered to be lost. Such particles are generally detected at the boundaries of the images where the particles move in and out of the field of view.

2.3 Sample and sample cell preparations

Below we describe in detail the preparation method to obtain the monodisperse colloidal samples used in this thesis. We used colloidal poly-N- isopropylacrylamide (pNipam) particles that are cross-linked microgel spheres. The spheres swell in water at room temperature, but shrink and undergo a reversible volume transition above the critical solution temperature [5-8]. In the swollen state, the density and refractive index match closely that of the solvent, preventing settling of the particles for several weeks and allowing to image particles deep inside the bulk of a dense suspension. The particles are labeled with a fluorescent dye that allows us to image them with LSCM.

2.3.1 Samples for crystal growth

For the colloidal crystal growth described in chapter 3, it is important to use a monodisperse suspension. In the following we describe the cleaning and preparation steps applied to obtain the final sample. The fluorescent Poly-N-isopropylacrylamide microgel suspensions were obtained from Zhibing Hu, University of North Texas. To screen the particle charge, LiCl 0.005 M was added to the suspension. The sample contained a small amount of larger particles (impurities) which were removed by centrifuging. To do this, the stock suspension was diluted five times with water, and was subsequently concentrated by centrifugation at a speed of 7500 rpm for 30 minutes. Because of the larger size, the impurities settled faster than the rest of particles. By taking out the upper half of the
Figure 2.4: An image of glass sample cell used for crystal growth. pNipam suspension (pink) is filled in the middle of the cell, covered by a microscope slide and glued by Bevat 2-hydroxyethylmethacrylaat (yellow).

In our experiments, we achieved a uniform pNipam colloidal suspension. In order to not waste the sample, the bottom half of the suspension was diluted and concentrated subsequently five times. To obtain the final sample, the monodisperse suspension was then centrifuged with a higher speed for a longer time (9000 rpm, 100 minutes) and subsequently water was removed with a pipette. The concentrated suspension was then diluted to an effective volume fraction of 55%.

We prepared a sample cell by making a hole of 10 x 2 x 1 mm in the middle of a 75 x 25 x 1 mm glass slide. To avoid contamination, the glass slide was soaked in chromic acid for one day, rinsed several times with Millipore water, and then dried in a vacuum oven. One side of the glass slide was closed by a microscope cover glass with dimensions 15 x 32 x 0.19 mm, glued by a dilute Bevat 2-hydroxyethylmethacrylaat solution. After curing by UV light for one day the cell was ready for use. We filled the suspension in the blank cell, which we covered by a microscope slide, sealed with 2-epoxy components glue. The final sample cell is shown in Fig. 2.4.

2.3.2 Samples for critical Casimir effect

The second part of the thesis (chapter 5-7) deals with the aggregation and phase transitions by critical Casimir forces. The samples for these studies were prepared by suspending the pNipam particles in a mixture of 3-Methyl Pyridine (3MP), water and heavy water. The range and amplitude of the force depends not only on the temperature, but also on the concentration of liquid components [9-11]. In this thesis, we investigate the temperature
dependence of the critical Casimir forces using two types of quasi two-component solvent. The first quasi two-component solvent consists of 3MP and water (H\textsubscript{2}O and D\textsubscript{2}O) with mass fractions of 0.25 and 0.75, respectively, and on the left side of the critical concentration $C_c = 0.31$. This solvent mixture separates into 3MP-rich and water-rich components upon heating to $T_{cx} = 52.2 \, ^\circ\text{C}$ (see Fig. 2.5) and will be used to investigate the colloidal gas-liquid transition in chapter 5. The other mixture has 3mp mass fraction of 0.28, and is closer to the critical point. This solvent mixture separates into 3MP-rich and water-rich components upon heating to $T_{cx} = 39.5 \, ^\circ\text{C}$. It will be used to investigate liquid nucleation and morphology of cluster aggregate in chapter 6 and 7. The change of solvent composition allows us to investigate the influence of the solvent composition on the particle pair potential (see chapter 5). pNipam particles with a weight fraction of ~0.3% are suspended in the binary solvent. The advantage of these particles is that their refractive index and density match closely that of the binary solvent, making the suspension transparent, and preventing particle sedimentation. We find that these particles are stable in the 3mp-water mixture over at least several months. The suspension is filled in a 0.2 x 4.0 mm clean glass capillary and the capillary flame sealed to avoid solvent evaporation.

2.4 Measurement of particle size

Particle volume fraction is the unique parameter controlling the crystal-fluid transition in hard sphere system. In our system, we tune the particle volume fraction by controlling the
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pNipam colloid size using temperature. In order to know precisely how the particle size changes with the temperature, we investigate the temperature dependence of the particle radius using dynamic light scattering techniques.

2.4.1 Sample preparation for dynamic light scattering

We diluted the monodisperse suspension to particle volume fraction of 0.5% by adding water. The suspension was then filled into a quartz cuvet and the cuvet was thoroughly closed by a cap and well winded by parafilm to avoid solvent evaporation. We embedded the cuvet in the scattering chamber, which was connected to a thermostat to control its temperature. We fixed the scattering wavelength $q = 0.37 \ \mu \text{m}^{-1}$ by fixing the scattering angle at $\theta = 90^\circ$. The incident laser beam has a scattering wavelength at $\lambda = 632 \ \text{nm}$. We measured the scattered intensity $I(t)$ and the reduced correlation function $g_1(q,t)$ as a function of time to determine the particle size.

2.4.2 Experimental results and discussion

We show the reduced correlation function at $T = 20 \ ^\circ\text{C}$ in Fig. 2.6a. The best exponential fit to the data gives the diffusion coefficient $D = 0.308 \ \mu \text{m}^2/\text{s}$. Using the viscosity of water at 20 \ ^\circ\text{C}, $\eta = 1.002 \ \text{Pas}$, and the Stokes – Einstein relation, we obtain the hydrodynamic radius of the pNipam particles $R_h = 694 \ \text{nm}$. We gradually increased the temperature from 20 \ ^\circ\text{C} to 40 \ ^\circ\text{C} in steps of 1 \ ^\circ\text{C}, and equilibrated the system for 30 minutes at each step before making a new measurement.

The resulting correlation functions of the pNipam suspension at different temperatures are shown in Fig. 2.6b. Single exponential functions are observed at all investigated temperatures. With increasing temperature, the correlation functions are shifted to the left indicating an increase in the diffusion coefficient, which reveals a decrease of the particle size with increasing temperature. The curves are clearly divided into two groups, indicating that the diffusion coefficient changes rapidly when the temperature increases from 32 to 33 \ ^\circ\text{C}. The resultant average hydrodynamic radius of the particles as a function of temperature is shown in Fig. 2.7 (solid triangle). The radius of the particle decreases from 694 nm at 20 \ ^\circ\text{C} to 345 nm at 38 \ ^\circ\text{C}, a decrease by a factor of 2. In detail, when the
temperature increases from 20 to 32 °C, the particle radius decreases slightly from 694 to 639 nm and it decreases rapidly when the temperature is in the range of 32-36 °C. However, the particle radius is almost constant when the temperature is above 38 °C. This is in good agreement with earlier observations [5-8].

We examine the reversibility of the particle size change by measuring the particle radius with decreasing temperature. The values are shown as open squares in Fig. 2.7. In general, both data sets are consistent. However, at 32, 34, and 36 °C the values show larger

![Reduced correlation function](image)

**Figure 2.6:** Reduced correlation function, measured by means of dynamic light scattering, of pNipam suspension at $\phi = 0.005$. (a) At $T = 20$ °C, experimental measurements (squares), and best single exponential (solid line). (b) At different temperatures, single exponential functions are observed at all investigated temperatures.

![Hydrodynamic radius](image)

**Figure 2.7:** Hydrodynamic radius of pNipam particle as a function temperature; solid triangle-measured with increasing of temperature, open square-measured with decreasing of temperature.
deviations. We associate these deviations with the rapid change of particle size at these temperatures. Here, a longer waiting time is needed for the system to reach the final thermal equilibrium.

### 2.5 Temperature-control setup

In this thesis, temperature is the key parameter controlling the colloidal phases. On the one hand, we use the temperature to tune the particle size to control the growth of large crystals, while on the other hand we use temperature to control the amplitude and range of the critical Casimir forces via fluctuations of binary solvents. The detail of the temperature-control setup is described below.

A photo and a schematic of the temperature setup are shown in Fig.2.8. A temperature stage is directly connected to a thermostat by a water loop. The thermostat controls the average temperature of the sample and the microscope objective with a stability of 0.02 °C. For the critical Casimir experiments (chapter 5, 6 and 7), the sample cell is mounted directly on the surface of the stage using thermal paste.

To guide the crystallization from its melt (chapter 3 and 4), we introduce a temperature gradient using peltiers. These peltiers are mounted on opposite sides of the temperature stage and they are placed upside down with respect to each other. A voltage is applied to both elements, the upper plate of one peltier warms up with respect to the water bath while the upper plate of the other peltier cools down. This results a temperature gradient from

![Figure 2.8](image_url)  
**Figure 2.8:** (a) An image of the temperature gradient setup and (b) its schematic configuration used for the crystal growth experiment.
left to right. A thin copper plate, 0.5 mm thick, is placed on top of the two peltiers in order to have a linear temperature profile. Finally, a sample cell is mounted on top of the thin cooper plate using thermal paste.

We use an infrared camera to check the temperature profile of our sample cell. An infrared image of the pNipam sample cell at an applied electric current of $I = 0.7$ A, and a voltage of $U = 3$ V is shown in Fig. 2.9a. Different colors in the image correspond to different temperatures (see the color scale on the right). This way, we measured the temperature across the sample cell as function of applied electric current. The resulting temperature profiles, for different electric currents at $U = 3$ V, are shown in Fig. 2.9b. The data confirms that, to good approximation, the temperature is a linear function of the distance.

**Figure 2.9:** Temperature gradient of sample cell: (a) an infrared-camera picture of the sample cell at $U = 3$ V and $I = 0.7$ A. (b) Temperature as a function of distance across the cell, measured with an infrared camera at $U = 3$ V and different applied electric currents to the peltiers.
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Bibliography