Manipulation and Imaging of Particles with Optical Tweezers and Confocal Microscopy

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BIOGRAPHY

Astrid van der Horst is a PhD student in the Soft Condensed Matter group at Utrecht University and the Bio-assembly and Organization group at AMOLF. After earning her bachelor degree in engineering physics and working in the radiotherapy department of the Netherlands Cancer Institute (NKI/AvL), Astrid van der Horst now focuses on the use of optical tweezers for precision positioning of and force measurements on colloidal particles.

ABSTRACT

3D micromanipulation and imaging of colloids has been achieved by combining optical tweezers and confocal microscopy using two microscope objectives. Arrays of up to several hundred time-shared traps have been created using acousto-optical deflectors. In addition, two axially separated trapping planes were addressed using a Pockels cell, making trapping and dynamic manipulation of 3D colloidal structures possible. Structures of high refractive index core-shell colloidal particles were trapped inside a concentrated dispersion of index-matched particles, thereby creating a nucleus for colloidal crystallization. Using confocal microscopy, the effects of such structures upon the non-trapped rest of the dispersion could be analysed quantitatively in 3D.

KEYWORDS

optical tweezers, confocal microscopy, core-shell, colloids, concentrated dispersions

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INTRODUCTION

Colloidal particles, with sizes ranging from a few nanometres to several micrometres, find their use in many fields, including chemistry and (bio-)physics. In suspension, their thermodynamic behaviour is similar to that of atoms and molecules, showing for example crystallization and other phase transitions [1,2]. Because of their size, colloidal particles, unlike atoms and molecules, can be imaged using (confocal) light microscopy [3]. In addition, the timescale on which processes take place allows for real-time analysis, and the size and morphology of the particles can be tuned. These features make colloidal suspensions suitable to serve as a condensed matter model system, or to make advanced materials such as photonic crystals.

Manipulating the position of individual particles using optical tweezers greatly expands the possibilities within these applications. Here we describe, after a short introduction on optical trapping, the use of optical tweezers to trap 2D and 3D arrays of particles. Then we demonstrate crystallization inside concentrated colloidal dispersions induced by the trapping laser beam. Finally we show the trapping of arrays of core-shell particles forming a nucleus for colloidal crystallization inside a concentrated dispersion. In addition, confocal microscopy is used to investigate the influence of such a well-defined structure on the bulk of the dispersion quantitatively in 3D.

OPTICAL TRAPPING

Since the first demonstration by Ashkin of the 3D single-beam gradient trap [4], the manipulation of particles with laser light has been used in a wide variety of fields, including chemistry, biology, and (bio-) physics. With this non-invasive technique, particles with sizes ranging from tens of nanometres to several micrometres can be manipulated. Dielectric particles, small metal colloids, and biological materials such as cell nuclei and bacteria can be trapped. The various applications include exerting and measuring piconewton-level forces [5], for example to study the stall force of molecular motors, using trapped particles as handles to manipulate non-trappable material like DNA, and manipulating particles directly, for example to pattern surfaces with colloids for epitaxial crystal growth [6].

In an optical tweezers setup, a strong light intensity gradient is created by focusing a laser beam to a diffraction-limited spot using a high-numerical aperture (NA) objective. For a particle with a refractive index higher than that of the surrounding medium, the force due to this intensity gradient is always directed towards the region with highest intensity, i.e. the focus. Particles with certain size and refractive index difference will be trapped in this potential well created by the focused laser beam.

To manipulate several particles individually, multiple traps can be created by time-sharing the laser beam [7]. The laser focus is rapidly scanned over several positions on a timescale shorter than the typical timescale of the Brownian motion of a particle inside the optical trap. This allows for 3D trapping of hundreds of particles using a single laser beam.

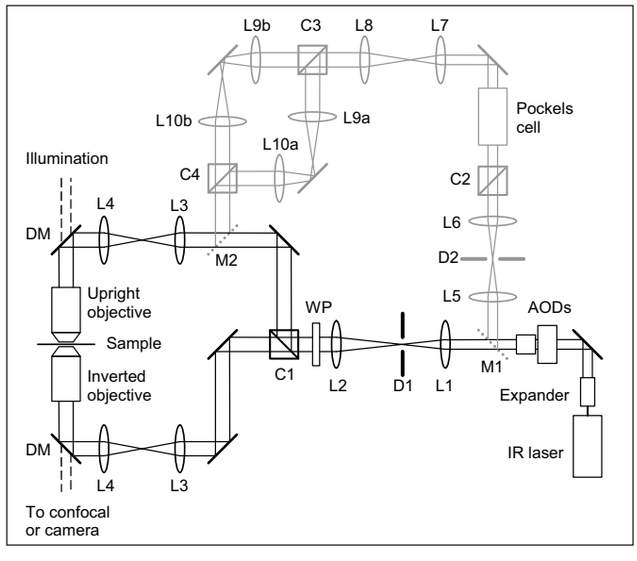

Figure 1:
Schematic of the optical tweezers setup. Polarizing beam splitter C1 sends the vertical polarized light to the inverted objective and the horizontal polarized light to the upright objective. The beam path used can be selected by rotating the polarization using half-wave plate WP. By inserting the mirrors M1 and M2 the laser can be sent to the Pockels cell and beam splitters C3 and C4 to create two trapping planes (beam path in grey).

MATERIALS AND METHODS

Core-shell colloids

To manipulate individual particles inside a concentrated dispersion, a mixture of in-house developed core-shell particles [8, 9] was used. The bulk of this colloidal dispersion consists of FITC-SiO_2 particles: 1.05 µm diameter silica (SiO_2) particles with a 0.4 µm core that is labelled with fluorescein isothiocyanate (FITC). The core can be imaged with confocal microscopy in fluorescence mode. The particles are suspended in a mixture of dimethylformamide (DMF) and dimethylsulphoxide (DMSO). The DMF/DMSO-ratio is chosen such that the refractive index n_D of this solution is matched at 1064 nm with that of silica (n_D = 1.47). Without a difference in refractive index with respect to the solvent, the silica particles will not be affected by the laser beam and can therefore not be trapped.

Added to this concentrated dispersion is a small amount of PS-SiO_2 core-shell particles. These colloids, 0.98 µm in diameter, have a 0.77 µm polystyrene (PS) core which can be imaged in reflection mode. Because the refractive index of PS (estimated n_D = 1.6) is higher than that of silica and of the DMF/DMSO-mixture, the particles can be trapped by the laser tweezers. The silica shell, of the same material as the FITC-SiO_2 particles, ensures equal interaction between all colloids in the solution.

Because of the matched refractive index of the solution, spherical aberration is minimized, and trapping and imaging deep into the sample are possible. By only imaging the cores, there is no overlap of the intensity profiles of adjacent particles. 3D co-ordinates of the positions of the particles can now be determined, making quantitative 3D analysis possible.

The other dispersions used consisted of 1.4 µm FITC-SiO_2 particles, with a fluorescent 0.4 µm core, either in ethanol (n_D=1.36) or in DMF (n_D=1.43).

Optical tweezers and confocal microscopy

In our optical tweezers system [8,9] an infrared laser beam (Spectra Physics, 1064 nm, 10 W cw) is strongly focused inside a sample on an inverted microscope (Leica, DM-IRB). A schematic of the setup is shown in Figure 1. Two high-NA objectives (Leica, both 100× or 63×, 1.4 NA, oil immersion) are used on either side of the sample to allow for trapping and simultaneous independent imaging [7]. Several trapping modes are possible (upright, inverted, and counter-propagating), while the sample can be imaged using bright field, differential interference contrast (DIC), epifluorescence or confocal microscopy. The wavelength of the trapping laser does not interfere with the excitation and emission of the fluorescent dyes, and, in addition, damage to biological materials is minimized.

Using the half-wave plate (WP) and polarizing beam splitter (C1), the beam can be directed either to the upper or to the lower beam path. With the laser beam entering from below, the inverted objective is used for both imaging and single-beam trapping, while the upright objective functions as a condenser. In this configuration, the trap will move with the lower objective and only one plane with respect to the trapping plane can be imaged. To decouple trapping and imaging, the trapping laser beam can be inserted through the upright objective. In this mode, the inverted objective is moved axially using a piezo-driven objective scanner (Physik Instrumente, P-721.20) so that a 3D confocal image set of the sample can be obtained. The upright objective still functions as a condenser for light microscopy. By inserting the laser via both objectives, a counter-propagating beam trap can be created, which is not shown here, but demonstrated in [8,9].

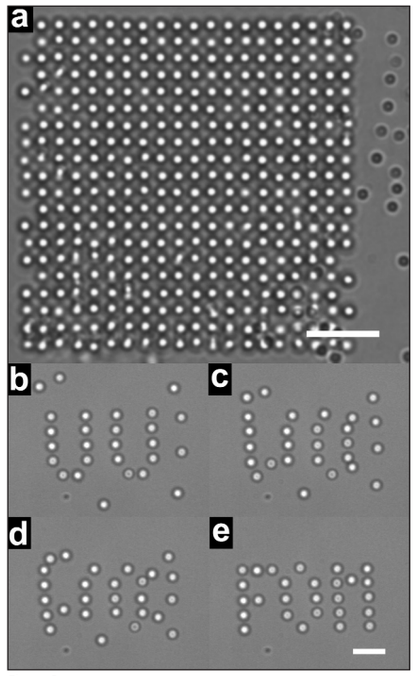

Figure 2:
Transmission microscopy images of 2D arrays of time-shared traps, filled with 1.4-µm diameter FITC-SiO_2 particles. From (b) to (e) the array was dynamically changed. Scale bars: 10 µm (a); 5 µm (b-e).

To control the lateral position of the trap inside the sample, two acousto-optical deflectors (AODs; IntraAction) are used. Inside the crystal of each AOD, a sound wave sets up a variable diffraction grating, which deflects the laser beam passing through. The frequency and amplitude of the sound wave determine the angle and intensity of the beam, respectively. The (1,1)-order, used for trapping and selected by the diaphragm (D1), can be deflected over a maximum angle of 30 mrad in both x and y directions. The AODs are positioned at a plane conjugate to the back focal plane of the objectives, and for a 100× objective this angle of 30 mrad corresponds to a displacement of the focus inside the sample of 28 µm. The positions addressed by the AODs can be changed at a rate of 220 kHz point-to-point to create an array of time-shared optical traps.

To ensure efficient trapping, the laser beam is expanded to overfill the back aperture of the objective. A 6× beam expander is placed directly after the laser, and lenses (L1, focal distance f = 120 mm; L2, f = 250 mm) expand the beam further. The expansion is done in two steps, because an expansion of the beam after the AODs decreases the lateral range within the sample, while the aperture of these modulators limits the expansion before the AODs.

In both the lower and the upper beam path, a telescope, formed by two lenses (L3 and L4, both f = 80 mm), sends the beam to a dichroic mirror (DM). These dichroics reflect the infrared trapping beam, but transmit visible light, allowing imaging of the sample. The lenses L3 are placed in a plane conjugate to the back focal plane of the corresponding objective. By moving these lenses in the x, y, or z directions, the focus of the laser inside the sample is moved accordingly.

By inserting mirrors (M1 and M2), the beam is steered, via a diaphragm (D2) and the telescope lenses (L5 and L6, both f = 120 mm), to the Pockels cell. In this electro-optical modulator (EOM; Conoptics) the polarization of the linear polarized laser beam is rotated over a chosen angle by changing the birefringence of the crystal inside.

In combination with polarizing beam splitter cubes (C4 and C5), the EOM can rapidly switch the beam between the two paths a and b. In both these paths, the lenses (L9 and L10, f = 90 mm, for all four lenses) form a telescope. The divergence of one path is changed by moving a lens (L9a) along the beam, creating two trapping planes axially displaced with respect to one another.

By synchronizing the AODs and the EOM, an array of traps can be created in each plane, arranging particles in a 3D structure. With the upright objective used for trapping, the inverted objective can be used for independent 3D imaging of the colloidal dispersion.

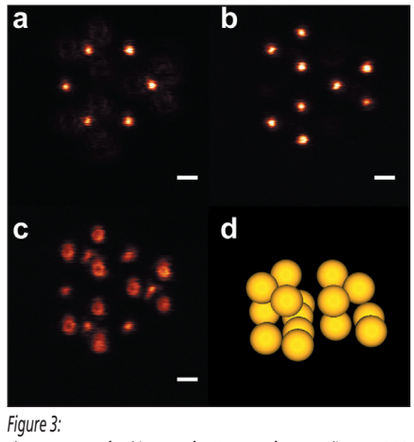

Figure 3:
Fluorescence confocal images of a 3D array of 1.4-µm diameter FITC-SiO_2 particles in ethanol (a-c), and a calculated reconstruction (d). The two trapping planes were 1.7 µm apart. Scale bars: 1 µm.

RESULTS AND DISCUSSION

Dynamic arrays of optical tweezers

Figure 2 shows brightfield microscopy images of time-shared multiple traps. In Fig. 2a an array of 400 traps was filled with FITC-SiO_2 particles. The particles were dispersed in ethanol inside a 100 µm-thick capillary. The inverted objective (63×, 1.4 NA) was used for trapping as well as for imaging. The laser power used was 1.0 W at the back focal plane of the objective, and the pattern was scanned by the AODs at a rate of 96 Hz.

In Figure 2b, 27 FITC-SiO_2 particles were trapped, of which 20 particles spell 'UU'. The array was then dynamically changed in eight computer-programmed steps, of which two are shown (Fig. 2c and 2d), to finally spell 'FOM' (Fig. 2e). This rearrangement took less than a second. The inverted objective (100×, 1.4 NA) was used for both trapping and imaging.

Trapping in two planes using the upright objective

In Figure 3 the inverted objective was used for fluorescence confocal imaging, while the upright objective was used for trapping in two axially displaced planes (both objectives 100×, 1.4 NA). The two trapping planes were 1.7 µm apart. Two 2D arrays of FITC-SiO_2 particles formed a 3D structure in ethanol. Six particles were trapped in the upper plane (Fig. 3a) and nine in the lower plane (Fig. 3b). Because of the extent of the point spread function in z, fluorescence from all cores was visible in Fig. 3c, the plane between the two trapping planes. From the 3D confocal image set, the co-ordinates of the particles were determined and were then used for a calculated reconstruction of the trapped structure (Fig. 3d).

Laser-induced crystallization in concentrated colloidal dispersions

Figure 4 shows fluorescence confocal images of FITC-SiO_2 particles. Imaging was done with the inverted objective (100×, 1.4 NA) and only the cores of the particles are visible. The colloids formed a sediment at the bottom of the 10 µm-thick sample.

In the absence of a laser beam, this sediment was in a liquid-like state (Fig. 4a), but when focusing the laser in the bottom layer using the upright objective (100×, 1.4 NA), the gradient force induced the formation of a crystallite (Fig. 4b).

Under different experimental conditions (higher laser power, DMF as solvent in a different sample cell), a larger crystalline region formed in the bottom layer, of which a section is shown in Figure 4c. Above this layer, a cone of particles formed along the optical axis of the laser. In Figure 4d, taken 4.3 µm above the bottom layer, this cone is visible as a ring of particles around the optical axis. A scan of the sample perpendicular to the bottom layer is shown in Figure 4e.

Selective trapping in concentrated dispersions

To study colloidal crystallization in a more controlled way, we looked at the effects of trapped configurations on the structural aspects of a concentrated mixture of core-shell particles in a DMF/DMSO solution.

In Figure 5 the combined fluorescence confocal images (green) and reflection confocal images (red) are displayed, averaged over several images (with a time-lapse of several seconds). Moving particles show up blurred in these time-averaged images, whereas immobile colloids appear bright. Only the cores were imaged, using the inverted objective, while trapping was done with the upright objective (both 100×, 1.4 NA).

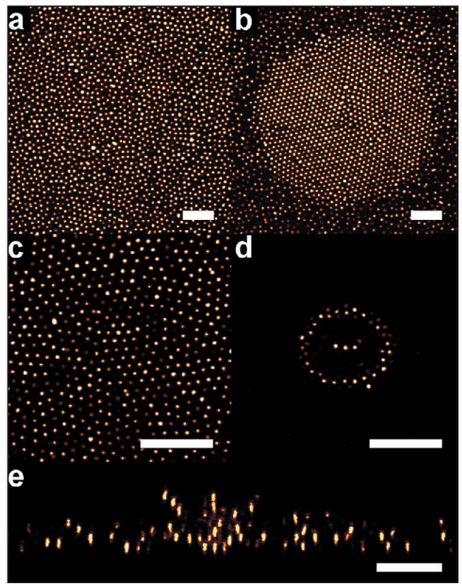

Figure 4:
Fluorescence confocal images of 1.4-µm FITC-SiO_2 particles, with the laser off (a) and on (b-e). (a-d) are x-y scans, and (e) is an x-z scan. Scale bars: 10 µm.

Eight PS-SiO_2 particles (red) were trapped in a 3 × 3 array of optical traps. Because the solvent was refractive index-matched with silica, the FITC-SiO_2 particles (green) were not influenced by the trapping laser, as can be seen at the position of the empty ninth trap. Here, the FITC-SiO_2 particles were neither expelled, which would show up as a darker spot in these time-averaged images, nor trapped, in which case the position of the ninth trap would appear bright.

In Figure 5a the array of trapped particles induced order in the dispersion, and in Figure 5b the dispersion in the array crystallized with a hexagonal symmetry. For an array with smaller lattice spacing, the induced ordering had a square symmetry (Fig. 5c). Figure 5d shows an array of particles trapped inside a crystalline layer with different lattice spacing, demonstrating that it is possible to trap and manipulate particles inside a colloidal crystal.

CONCLUSIONS

The combination of optical trapping and confocal microscopy is a powerful tool to study colloidal phase transitions. Individual particles can be manipulated and independently imaged in 3D with the use of two microscope objectives. The AODs enable the creation of large and dynamically changeable arrays of time-shared optical traps. Synchronization of the AODs with a Pockels cell yields 2D arrays in two separate planes forming a 3D structure using a single microscope objective. With a system of core-shell particles, individual particles can be trapped and manipulated inside a dispersion of refractive index-matched particles, which experience no trapping forces. The templates of trapped particles act as crystallization nuclei and induce structure in concentrated dispersions. By imaging the cores of the particles using confocal microscopy, the effects of a trapped structure on a concentrated colloidal dispersion can be studied quantitatively, in a controlled way, in three dimensions

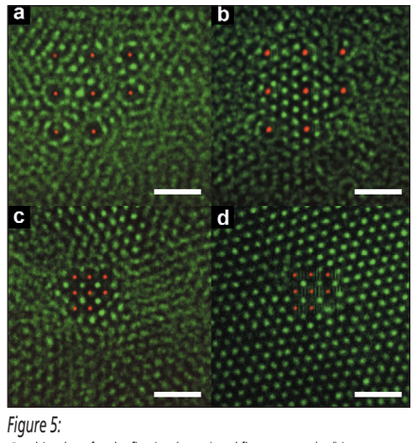

Figure 5:
Combined confocal reflection (green) and fluorescence (red) images averaged over several seconds, showing 8 trapped PS-SiO_2 particles in a dispersion of index-matched FITC-SiO_2 particles. The lattice spacing of the template was 4.1 µm (a-b), 1.6 µm (c), and 1.8 µm (d). Scale bars: 5 µm.

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