Speckle Tweezers for Simultaneous Controlling of Low and High Refractive Index Micro-particles

Ramin Jamali¹, Farzaneh Nazari², Azadeh Ghaffari³, Sabareesh K. P. Velu⁴, and Ali-Reza Moradi¹,⁵,*

¹Department of Physics, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan 45137-66731, Iran
²Department of Physics, Yazd University, Yazd 89195-741, Iran
³Department of Food and Drug Control, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan 45139-56111, Iran
⁴Department of Physics, Rathinam College of Arts and Science, Coimbatore 641021, Tamilnadu, India, Email: dean.rd@rathinam.in
⁵School of Nano Science, Institute for Research in Fundamental Sciences (IPM), Tehran 19395-5531, Iran
*moradika@iasbs.ac.ir

ABSTRACT

Manipulation of micro and nanoscale particles suspended in a fluidic medium is one among the defining goals of modern nanotechnology. Speckle tweezers (ST) by incorporating randomly distributed light fields have been used to apply detectable limits on the Brownian motion of micro-particles with refractive indices higher than their medium. Indeed, compared to periodic potentials, ST represents a wider possibility to be operated for such tasks. Here, we extend the usefulness of ST into low index micro-particles. Repelling of such particles by high intensity regions into lower intensity regions makes them to be locally confined, and the confinement can be tuned by changing the average grain intensity and size of the speckle patterns. Experiments on polystyrenes and liposomes validate the procedure. Moreover, we show that ST can be used to nano-particle (NP)-loaded liposomes. Interestingly, the different interactions of NP-loaded and empty liposomes with ST enables collective manipulation of their mixture using the same speckle pattern, which may be explained by inclusion of the photophoretic forces on NPs in NP-loaded liposomes. Our results on the different behavior between empty and non-empty vesicles may open a new window on controlling collective transportation of drug micro-containers along with its wide applications in soft matter.

1 Introduction

Using light to control the motion of micro- and nano-structured objects is a challenge and involves several scientific and technological fields such as optical tweezing⁴, Van der Waals and Casimir interactions⁵,⁶, integrated optics⁷, biophysics⁵, etc. However, in more complex light-activated devices the unavoidable disorder induces some effects including multiple-scattering, diffusion, and localization of light⁶–⁸. The disorder can be externally controlled, which leads to applications such as tunable lasers, transmission of light through random media, and novel disorder driven devices, such as ultrasensitive spectrometers⁷,⁸,¹⁰. Yet, there is a specific field -optomechanics- in which the beneficial features of randomness have not been sufficiently investigated. Optomechanical forces come from the interaction of the electromagnetic wave with the boundaries of dielectric objects. On the other hand, random systems include a large number of boundaries for which calculation of the forces is not trivial, but it is crucial to understand the mechanisms of optically activated devices¹¹.

Manipulation of micro and nanoscale particles suspended in a fluidic medium, in general, is one of the defining goals of modern nano-technology¹². The conventional strategy is to trap particles utilizing optical trapping forces¹, photo-acoustic effects¹³, and magnetic and electric forces¹⁴. Progressions into a specific approach has resulted in numerous breakthroughs in pharmaceutics and microfluidics¹⁵–¹⁷. A primary advantage of specific methodology is the opportunity for it to be applied in the manipulation of drug containers in targeted drug delivery¹⁸. Among various approaches, optical tweezers have been particularly prosperous due to their inherent versatility. Optical trapping of micron-sized particles was introduced by A. Ashkin¹⁹. Since then, it has been successfully implemented in two size ranges: the subnanometer scale for cooling of atoms, ions and molecules²⁰,²¹, and the micrometer scale for non-invasive manipulation of microscopic objects such as cells and bacteria²². Further developments through externally controlling the position and stiffness of individual trap sites, offer manipulation tasks in parallel and lead to many important breakthroughs in bioscience, materials science, microfluidics, and soft condensed matter physics²³. Conventional optical traps require careful engineering and aligning of setups and sample preparation. Therefore, such stringent conditions are not matchable with the simplicity, low-cost and high-throughput requirements that are necessary for biomedical applications.
The light gradient force exerted on a dielectric particle must overcome the scattering force for steady trapping, especially along the axial direction. The gradient force acting on the particle is proportional to $\pm \nabla E^2$, where $E$ is the electric field of the beam, and the difference in the refractive indices between the surrounding medium $n_m$ and the particle $n_p$ indicates the sign. The realization of stable trapping of high-refractive-index particles is easy since the intensity profile of tightly focused laser beams with a spatially homogeneous state of polarization is in the form of a Gaussian distribution. However, for low-refractive-index particles, i.e., when the refractive index of the particle is lower than that of the surrounding medium ($n_p < n_m$), the gradient force will point from the focus center to the region of low-intensity and will repel the particle away from the highest intensity point. Therefore, low-index particles require a different strategy to be manipulated by optical tweezers; to trap them, a beam with a focusing feature of hollow intensity distribution, the so-called hollow beam, is used. Various types of hollow beams, such as high order Bessel beams, Laguerre-Gaussian beams, azimuthally polarized beams, and the output beam of small hollow fibers, etc. have been tested and applied for this purpose in recent years. Trapping by hollow beams, furthermore, results in a reduction of possible photo-damage, which is crucial for biological specimens.

On the other hand, it is known that low-refractive-index particles have an important role in physics, medicine, and technology. For example, trapping a single gas bubble in water in an acoustic resonant cavity has allowed several studies to understand the physics of sonoluminescence and to establish further applications in biology and medicine. More importantly, the bubbles are used to enhance the contrast of images in ultrasound imaging. Besides bubbles, low-refractive-index particles are found in water-in-oil emulsions for petroleum, and food processing applications. Another important class of low-index particles are liposomes. A liposome is a spherical vesicle with a molecularly thin and self-assembled membrane, and may act as an appropriate container for nutrients and pharmaceutical medicines in drug delivery and can be applied to enhance the transfection of genes into living cells. Liposomes are made of phospholipids and are developed by disrupting biological membranes, their most important kind being the multilamellar vesicles.

Usually, it is difficult to optically trap both kinds of particles (low and high-refractive-indices) as it requires complex scanning methods and/or rigorous design of holographic tweezers. Therefore, conventional methods are not suitable for more complex micro-manipulation tasks such as transportation of a large number of such mixtures of particles. Also, limitations such as the short working distance of high numerical aperture microscope objectives undermine the efficiency of the methods. In this paper, we propose the use of speckle tweezers (ST). ST possesses both the simplicity and applicability in optical manipulation of real-life situations. Speckle light patterns are high-contrast, fine-scale granular patterns that are the result of the interference of a large number of dephased but coherent monochromatic light waves propagating along different directions. Speckle patterns can be generated through different processes, such as scattering of laser light from a rough surface or mode-mixing in a multimode fiber. It has been demonstrated that ST is a versatile tool to efficiently perform collective optical manipulation tasks, such as trapping, guiding, and sorting of high-refractive-index particles. Although, the intrinsic randomness of speckle patterns is considered to be a disturbing noise in experiments, nevertheless, the patterns find several useful applications. One of the first experiments on the use of ST in this range demonstrated the emergence of anomalous diffusion in colloids and showed the control of the motion of Brownian particles. Recently, the optical speckle field has been used for producing a thermal speckle field through interaction with plasmonic substrates and converting the high-intensity speckle grains into the corresponding thermal speckle grains. In another recent study, it has been shown that the density of the structural defects in a 2D binary colloidal crystal can be engineered using a speckle field. A memory equation based on a theoretical model was provided to describe the motion of colloidal particles within the ST field. The speckle field contains a random, but somehow tunable, distribution of isolated high-intensity regions surrounded by irregular and interconnected low-intensity regions, and the dark spots are, indeed, much more common than bright ones. This nature of the speckle fields makes the use of ST as an elegant possibility for collective manipulation of mixtures of low and high index micro-particles. Regions with higher and lower intensities in the speckle field can be used to confine the high-refractive-index and low-refractive-index particles, respectively. Moreover, by examination of speckle fields effect on not-empty but nano-particle (NP) loaded liposomes, we experimentally corroborate that the ST may be used to perform control on the collective movement of drug micro-containers.

2 Materials and methods

2.1 Sample preparation

Liposomal of clindamycin phosphate (CP) is prepared by thin-film hydration method and characterized in terms of size, size distribution, zeta potential, stability, and encapsulation efficiency. CP is purchased from Suzhou Pharmaceutical. Monobasic potassium phosphate (98–100.5%), phosphoric acid (85–88%), sodium hydroxide (>98%), Tween 80 (for synthesis), triton X-100 (99%) and high performance liquid chromatography HPLC-grade acetonitrile are purchase from Merck. Sephadex G-50 is purchased from MilliporeSigma. Egg phosphatidyl cholin (E80) is purchased from Lipoid. Cholesterol (99%) is purchased from Sigma-Aldrich. The lipid mixture is dissolved in chloroform/methanol (2:1) and get dried under a reduced pressure in a rotary evaporator at 60°C to form a thin lipid film. The film is then hydrated with CP solution (63 mg/ml) in phosphate buffer.
Figure 1. (a) Schematic of the experimental setup; BE: beam expander, MO: microscope objective, MF: multi-mode fiber, NDF: neutral density filter, BS: beam splitter, FC: fiber coupler, L: lens, M: mirror. (b) A bright field microscopy image of the speckle pattern at the sample plane. Inset: Intensity distribution of speckle pattern. Blue bars demonstrate the experimental data and red solid line shows the theoretical probability density function, which follows the negative exponential distribution. (c) Normalized speckle pattern intensity spatial auto-correlation function $I_s(r)$ as a function of position $r$. The blue and red lines are speckle intensity along x and y axis, respectively, and the dashed blue and red lines are their corresponding Gaussian functions. The average speckle grain size is determined from the standard deviation of the Gaussian function. In our case, the average speckle grain size is 1.69 ± 0.20 µm.

The liposome size can vary from very small (0.025 µm) to large (2.5 µm) vesicles. Moreover, liposomes may have one or more bilayer membranes. The vesicle size is an acute parameter to determine the circulation half-life of the liposomes. We separate out the liposomes of 1 µm range by passing them through syringe filters (Sterlitech). These liposomes are in multilamellar vesicles class, i.e., their structures consist of concentric phospholipid spheres separated by layers of water. The refractive index of the formed liposomes is 1.300, which is measured by Abbe refractometer (Atago, NAR-1T).

Gold NPs of 100±5 nm diameter and optical density of 1, and polystyrene particles of 1 µm diameter are purchased from Sigma-Aldrich. Citrate buffer, which is a proprietary surfactant is used as a stabilizer for NPs. Gold NPs are loaded passively to the liposomes i.e. encapsulated during the liposome formation. To this end, during the liposome preparation and in the hydration stage, 100 microliter of gold NPs are added to distilled water. Before the experiments, polystyrene particles, liposomes, and the NP-loaded liposomes are shaken for a few minutes by an ultrasonic bath (Xuba3, Grant), and separate dilute
solutions of which, are suitable for trapping experiments, are prepared. The concentration could be further adjusted by the use of a push-pull syringe pump, connected into the surrounding medium and the high concentrate liposome reservoirs. For example, for increasing the concentration, we infuse the liposomes directly into the chamber and withdraw the surrounding medium at the constant rate.

We fabricate special chambers with a couple of glass coverslips (22 × 22 mm, thickness of 170 ± 5 µm) and parafilm spacers, in which parts of one of the building coverslips are coated by a semi-transparent layer, for reducing the transmittance of the laser light up to 15%. The coating is performed using Sliver (Ag) sputter targets in a sputter coater system (DST3-A, Nanostructured Coatings Co.) on the pre-covered areas of the coverslip. By tuning the coating duration and the applied voltage the transmittance of the coated area can be tuned.

2.2 Experimental Procedure

Figure 1a shows the schematic of the home-built STs experimental setup to perform the experiments. A laser beam (DPSS, 85-GSS-309, Melles Griot, 532 nm) is expanded by the beam expander (BE) and by the use of a microscope objective (MO1, 10×, NA = 0.25) is focused onto the input entrance of a multi-mode fiber (MF). A step-variable neutral density filter (NDF) is used to reduce the beam intensity in steps. A beam splitter (BS) is also used to direct a white light illumination along with laser light into the sample for conventional microscopic imaging. The multi-mode fiber has a core diameter of 365 ± 14 µm, numerical aperture of NA = 0.22, and core and cladding refractive indices of 1.4589 and 1.4422, respectively. Therefore, our MF can mix up to approximately 474 modes of the propagating laser beam, and the output light from the fiber is the interference of several modes with random phases and results in generating the speckle pattern at the sample plane. The microscope objective MO2 (40×, NA = 0.65), mirror (M), and the lens (L2) form the image of the sample on the camera (DCC1545M, Thorlabs, 5.2 µm pixel pitch). The sample chamber consists of a microfluidic channel with an inlet and outlet to inject, withdraw, or flow aqueous samples by the use of a syringe pump (NE-300, Just Infusion™).

Figure 1b is a typical speckle pattern as observed on the camera. The average grain size of the speckle field in this pattern is 1.69 µm and the average speckle intensity is \( \langle I \rangle = 200 \text{mW/} \mu \text{m}^2 \). In its inset the theoretical (red color solid line) and experimental (blue color bars) probability function of the speckle pattern intensity is shown. The experimental data is obtained by averaging the intensity distribution of 1000 speckle patterns. The probability density function of the speckle pattern intensity follows the negative exponential distribution, i.e., \( \frac{1}{\langle I \rangle} \exp \left( \frac{I}{\langle I \rangle} \right) \). Figure 1b shows a good agreement between theory and the distributions of the speckle pattern intensities used in the experiments, which confirms that the speckle patterns used in the experiments are well-formed.

Figure 1c shows the auto-correlation function of the speckle pattern intensity. The average speckle grain size is determined by evaluating the normalized spatial auto-correlation function \( I(r) \) on the 2D imaged speckle patterns. The resulted normalized auto-correlation function is fitted by the Gaussian function, whose standard deviation is approximately one-third of the average speckle grain size.

3 Results and Discussion

To demonstrate trapping and the manipulation of low-refractive-index particles using a speckle field we start by considering the motion behavior of two samples containing polystyrene microspheres (diameter of 1 µm, refractive index of \( n_p = 1.59 \)) and liposome vesicles (diameter of 1 µm, refractive index of \( n_l = 1.3 \)) under the exposure of randomly distributed laser field. We brought the output end of a multimode fiber into the specimen chamber to impose the speckle field on the dispersed particles. The average intensity of the speckle grains is controlled by tuning the intensity of the laser light, and the grain size can be varied by careful adjustment of the fiber end to sample distance. The microfluidic chamber is connected to an infusion pump to infuse the micro-particles and allow them to flow (infusion rate=1.25 ml/hr). First, in separate experiments, we imposed the speckle field on multiple low- and high-refractive-index particles in a quiescent medium \( (n_m = 1.33) \). Figures 2a and 2b show the path trajectories of the typical low- and high-refractive-index particles, respectively, for the different speckle field strengths. As the trajectories show, polystyrene beads reposition themselves in the bright regions of the speckle pattern and under strong optical forces \( \langle I \rangle = 200 \text{mW/} \mu \text{m}^2 \) they remain trapped in one of the speckle bright grains for several minutes. However, according to their Brownian motion, the particles can also move inside the speckle grain. By reducing the average laser intensity to \( \langle I \rangle = 100 \text{mW/} \mu \text{m}^2 \) they are still trapped but can move between the neighboring grains. By further reducing the average intensity \( \langle I \rangle = 10 \text{mW/} \mu \text{m}^2 \) the optical forces become relatively low and the particles may show almost free diffusion but still feel the speckle field. Similar behavior is observed in liposome vesicles. However, predictably, according to their low-refractive-index with respect to their surrounding medium, as the trajectories of Fig. 2b show, the liposomes are restricted to the dark regions with high laser intensity and move between few neighboring dark regions with lower intensity. Eventually when the average intensity reduces to \( \langle I \rangle = 10 \text{mW/} \mu \text{m}^2 \) the liposomes can move between several dark regions. The optical force vector acting on a moving particle moves in the speckle field and varies with a characteristic time scale, which is inversely proportional to the average speckle intensity in the first approximation \(^{41}\). The motion of a Brownian particle in a
Figure 2. The experimental trajectories (turquoise solid lines) of high and low-refractive-index particles in regressive confinement through decreasing the average speckle intensity ($\langle I \rangle = 200 \text{ mW}/\mu\text{m}^2$, $\langle I \rangle = 100 \text{ mW}/\mu\text{m}^2$, $\langle I \rangle = 10 \text{ mW}/\mu\text{m}^2$). The surrounding medium is distilled water of refractive index $n_m = 1.33$, viscosity of $\eta_w = 0.001 \text{ Ns/m}^2$ at temperature $T = 300 \text{ K}$. The background represents the speckle field generated by propagating of a solid state laser light through a multimode fiber. (a) Trajectories of a polystyrene particle of diameter $D_p = 1 \mu\text{m}$ and refractive index of $n_p = 1.59$. (b) Trajectories of a liposome vesicle of diameter $D_l = 1 \mu\text{m}$, refractive index of $n_l = 1.3$, and viscosity of $\eta_l = 340 \times 10^{-3} \text{ Ns/m}^2$.

The mean square displacement (MSD) of the particle motion is a quantitative expression to describe the aforementioned behavior of the particle inside the speckle field, and is defined as $\text{MSD} = \langle \Delta r^2(\Delta t) \rangle = \langle [r_{j}(t_0 + \Delta t) - r_{j}(t_0)]^2 \rangle_{j,t_0}$, where $r_{j}(t)$ denotes the position of micro-particles $j$ at time $t$, and the average is derived over all micro-particles $j$ and all onset time $t_0$. It means that, we consider both the ensemble and time average. We obtain each MSD curve by taking an average over 1000 different trajectories of the micro-particles we have used (polystyrene, empty liposome and NP-loaded liposome). Figures 3a, 3b, and 3c show the MSD of a polystyrene particle, a liposome, and a liposome containing NPs, respectively, that are moving in a static speckle field of different average speckle intensities. In the absence of optical forces or for low optical forces and for longer times, the MSD behaves almost linearly with $\Delta t$ ($\text{MSD} \sim 4D_{SE} \Delta t$, where $D_{SE}$ is the Stokes-Einstein diffusion coefficient). As the forces increase and $\tau$ decreases, there is a transition into a subdiffusive regime characterized by $\text{MSD} \propto \Delta t^\beta$ with $\beta < 1$. For a very large $\Delta t$, the motion returns into the diffusive regime, i.e., $\beta = 1$, with a smaller effective diffusion coefficient $D_{\text{eff}}$. Under higher external speckle forces, after the waiting time, the particle takes a rest time until bounded in another speckle.
Figure 3. Mean square displacement of Brownian particles on a logarithmic scale as a function of time in increasing speckle field intensity, for (a) a polystyrene, (b) a liposome, and (c) a NP-loaded liposome. Black lines represent Einstein’s free diffusion law. The initial position of the particles is chosen randomly and the reported data in each of the figures are the average of more than 1000 trajectories in a time duration of 1000 s. The average speckle intensity is increased from 10 to 200 mW/µm². In all cases, a transition from trapping at high speckle intensities to subdiffusion at low speckle intensities can be recognized.

To demonstrate the acting optical forces on a low-refractive-index particle in a speckle light field, we obtain the 2D microscopic force fields through force reconstruction via the maximum-likelihood-estimator analysis (FORMA) algorithm in its 2D form, which is introduced in. In FORMA the forces are retrieved by the analysis of the particles’ Brownian trajectories. The optical force fields generated by speckle patterns are larger extended force fields and the equilibrium positions are not known a priori due to their random appearance and include non-conservative components. FORMA estimates accurately the conservative and non-conservative components of the force field simultaneously with important advantages over the common methods that obtain the forces by analyzing their influence on the particles’ Brownian motion. This method is more accurate, does not need calibration fitting parameters, executes much faster, and requires much fewer data. In Figs. 4a and 4b the results of the force field reconstruction in the speckle field are shown for the high- and low-refractive-index particles, respectively. The green color background is the overlaid images of the speckle field pattern, whose intensity is proportional to the potential depth of the optical potential felt by the micro-particles. The red arrows plot the 2D force field exerted on the micro-particles and measured with FORMA. The trajectories (thin turquoise lines) are acquired experimentally for the motion of a polystyrene particle of diameter $D_p = 1 \mu m$ and refractive index of $n_p = 1.59$ (Fig. 4a) and liposome vesicle of diameter $D_l = 1 \mu m$, refractive index of $n_l = 1.3$, viscosity of $\eta_l = 340 \times 10^{-3} \text{Ns/m}^2$ (Fig. 4b) in distilled water of refractive index $n_m = 1.33$, viscosity of $\eta_m = 0.001 \text{Ns/m}^2$, and at temperature $T = 300 \text{K}$. As illustrated in Fig. 2, a polystyrene (a liposome) explores the high (low) intensity regions while being trapped for a while in each bright (dark) grains before crossing over a neighbor one.

We have verified these results on simulated data considering the same physical parameters of the experiments (Figs. 4c and 4d). The results of our simulations are in very good agreement with the results of the experiments. The motion of a Brownian particle in a static speckle field without fluid flow is the result of random thermal forces and the gradient optical forces, which attract high-refractive-index particles towards the intensity maxima of the optical field and, on the contrary, repel the low-refractive-index ones to the intensity minima. The motion of a Brownian particle in presence of an external potential, such as a speckle pattern, can be modeled by solving the Langevin equation, in which the inertial effects in the motion of the particle are usually neglected. Also, the influence of the particles in the speckle field is neglected because of the low concentration of the immersed particles. The force fields of the simulated data are obtained through the ray optics approach. The details of the force fields of the high- and low-refractive-index simulated particles (Figs. 4c and 4d) moving in speckle fields show very good agreement with the experimental data. As it is shown, the magnitude of the acting force vectors on the high-index particles is comparable with the one of low-index particles in any typical point within the speckle field, both in experimental (Figs. 4a and b) and simulated (Figs. 4c and d) force fields.

In, G. Volpe et al. demonstrated that by employing a time-varying speckle pattern and via continuous control of the subdiffusion (a slope smaller than 1 in the MSD(Δt) function) to superdiffusion (a slope bigger than 1) transitions, it is
possible to perform optical manipulation tasks such as guiding, sieving and sorting multiple particles. Indeed, compared to periodic potentials which are characterized by a few potential depths, ST represents a wider possibility (due to a distribution of intensities) to be operated for such tasks, provided that a controlling handle exists. However, controlling the variation of a produced speckle pattern comes along with challenges due to the inherent randomness of the speckle light fields. Nevertheless, the optical forces exerted on a particle depend on the particle’s physical parameters, hence, a static speckle pattern can be employed to realize a selection sieve in the presence of flow.

A significant observation in this work is that for a flow of low-index particles e.g. liposomes, the immediate effect of the presence of a speckle field is the reduction of their drift velocities. More importantly, for the liposomes inside which we loaded gold NPs, we observe that the reduction of the velocities is substantially reduced. For these experiments, we fabricated special chambers in which parts of one of the building coverslips are coated by a semi-transparent thin layer, therefore, it rejects partly the incident light. The rest of the uncoated area transmits almost all the light. In the chamber shown in Fig. 5a the right half of the coverslip is coated while in the one shown in Fig. 5b only the central part remains uncoated. Then a mixture suspension of empty liposomes and NP-loaded liposomes are injected into the chamber under a constant rate via a syringe pump and the flow is live monitored. Gold color and red dots represent the NP-loaded and empty liposomes, respectively. In Fig. 5, for both of the two aforementioned chambers it is demonstrated that microfluidic speckle filtering of NP-loaded liposomes from the empty ones is possible in a flow duration of a few seconds and within less than 50 µm range. The separation can be done because the
Figure 5. Microfluidic speckle filtering of NP-loaded liposome (gold color dots) from the empty ones (red dots), based on their velocity hindering in varying speckle intensity. The average speckle intensity is changed (from $\langle I \rangle = 300$ mW/µm$^2$ to $\langle I \rangle = 45$ mW/µm$^2$, or vice versa) in (a) one, and (b) two boundaries. Sample time lapses belong to every 2 s and the process takes a time duration of about 8 s.

NP-loaded liposomes drop behind with respect to the empty ones. Figure 5a shows that the presence of regions with different average intensities can tune the speed reduction difference, and, therefore, can boost the separation of the two sets of particles at the boundary of the regions. We demonstrate this type of control on the speed reduction difference further in Fig. 5b by adding another boundary which enables further control on the collective behavior of the flowing particles. This possibility to apply local changes on the average intensity over the speckle field makes it possible to design useful arrangements for directed collective motion of multiple particles.

In order to express the results in a more quantitative fashion, we track several particles during their flow and measure their velocities. Figure 6 summarizes the results. As shown in Fig. 6a, in case I by coating a semi-transparent layer on a half of the chamber surface to reduce the transmittance of the laser light by 85%, two regions with average speckle intensity of $\langle I_R1 \rangle = 300$ mW/µm$^2$ (Region 1) and $\langle I_R2 \rangle = 45$ mW/µm$^2$ (Region 2) are achieved. In case II three regions of different average speckle intensity, $\langle I_R1 \rangle = 45$ mW/µm$^2$ (Region 1), $\langle I_R2 \rangle = 300$ mW/µm$^2$ (Region 2), and $\langle I_R3 \rangle = 45$ mW/µm$^2$ (Region 3) are existed.

Figures 6b,c and 6d-f show the average velocity of 50 particles of each type for the first 8 s of experiments for the case I and case II, respectively. Blue dots and red squares indicate the velocities of empty liposomes and NP-loaded liposomes, respectively. We also conduct a reference experiment, in which the laser is off and the speckle field is removed, therefore, the liposomes have the constant flowing speed along with the fluctuations caused by Brownian motion (black diamonds). It is obvious that both types of particles encounter velocity reduction. However, the reduction is substantially different for the NP-loaded and empty liposomes. The reduction is also different in different regions. These outcomes are shown by the difference in the slopes of the fitted lines to the data. In Fig. 6b the lines fitted to velocities of the empty liposomes (blue line) and the NP-loaded liposomes (red line) in the initial seconds (moving in Region 1) have the slopes of $m_{L,R1} = -0.84667$ µm/s$^2$ and $m_{NP,R1} = -1.2167$ µm/s$^2$, respectively. When the particles cross the boundary of the two regions of case I (Fig. 6c) the decrease of the velocities of both types in Region
Figure 6. (a) Case I: the chamber surfaces is half-coated by a semi-transparent layer to reduce the transmittance of the laser light by 85% in Region 2, and Case II: two-third of of the chamber surface is coated (Regions 2 and 3). (b) and (c) The average velocity of empty (blue dots) and NP-loaded (red squares) liposomes and their associated fitted lines in Regions 1 (panel b) and 2 (panel c) of case I. (d-f) The average velocity of empty and NP-loaded liposomes and their associated fitted lines in Regions 1 (panel d), 2 (panel e) and 3 (panel f) of case II. The slope of the fitted lines are in $\mu$m/s$^2$ units. The black diamonds represent the velocity of a liposome when the speckle generating laser is off, as a reference experiment.
2 follows a different trend \( (m_{L,R2} = -0.3 \mu m/s^2 \) and \( m_{NP,R2} = -0.38429 \mu m/s^2 \). For the case II the trends of the same order in velocity reduction of empty and NP-loaded liposomes are measured in the Regions 1 and 3, which have similar average speckle intensity \( (m_{L,R1} = -0.34 \mu m/s^2, m_{L,R3} = -0.196 \mu m/s^2 \) and \( m_{NP,R1} = -0.536 \mu m/s^2, m_{NP,R3} = -0.301 \mu m/s^2 \). However, the velocity in Region 2 which has higher average speckle intensity decreases dramatically \( (m_{L,R2} = -0.99143 \mu m/s^2 \) and \( m_{NP,R2} = -1.1844 \mu m/s^2 \).

It is obvious that more than 30% of the velocity reduction difference between the empty and NP-loaded liposomes in a few seconds, which is deduced from our results, is sufficient to separate out the two important classes of the particles. The separation can be followed by guiding the first outgoing particles into a specific reservoir as no NP-loaded ones. Proper geometry of the fluidic channels and controlled speckle field engineering can be also combined toward the application of the method in endoscopic studies. It is remarkable that the hydrodynamic interactions between dispersed particles induced with the speckle field, cause the changes in their particle-particle distances and can hinder their diffusion\(^{54}\). However, this effect is common for either NP encapsulated or empty liposomes and can be disregarded in the presented experiment schemes.

The difference between the velocity reduction of NP-loaded and empty liposomes may be explained by the inclusion of the photophoretic forces on NPs. Photophoretic force is the result of thermal processes caused by the absorption of laser light by the particles. Therefore, in the optical force calculation for metallic NPs, either entrapped inside bubbles or freely moving, the effect of the local electromagnetic fields and the photophoretic forces have to be considered\(^{55}\). The theoretical investigation of the applied forces for irregularly shaped particles or irregularly distributed laser fields can be performed by the T-matrix method\(^{56}\). However, quantitative consideration of the photophoretic force on an absorbing particle involves many factors, such as pressure, light properties e.g. beam profile, intensity, wavelength, and polarizations, and particle properties e.g. size, geometrical shape, and thermal conductivity\(^{57,58}\). The effects can be either a pushing or pulling force. It has been already shown that airborne metallic NPs may be trapped in the dark regions of speckle fields\(^{55}\). Here, similarly, the photophoretic trapping partially holds the NPs, therefore, and it hinders the associated liposomes velocities.

Gold NP, itself, can enter liposome and be used as a drug. Moreover, NP-loaded liposome can resemble drug-loaded containers in drug delivery, in general. Therefore, this platform along with easy handling of speckle fields can provide an elegant approach for controlling the drug-loaded containers, for example for collective manipulation of transfection reagents, cell markers, and carriers of molecules\(^{59}\).

### 4 Conclusions

In conclusion, we extend the usefulness of speckle tweezers into low refractive index micro-particles and reported different particle speckle field interaction between empty and nano-particle loaded vesicles. The speckle fields have great potential to collectively guide, select, trap, or push micro-particles of low- and high-refractive-index. The high-intensity regions of the speckle fields can act as a size selection sieve for dielectric particles of high-refractive-index. On the other hand, and more importantly, the low-intensity regions of the speckle fields can perform the aforementioned tasks through the size selection sieve for dielectric particles of low-refractive-index, or through a photophoretic sieve for absorptive ones, whether embedded inside the containers or not. The rather low average intensity throughout the field that illuminates the samples can be considered as another profitable feature of the methodology. Furthermore, in addition to the inherent irregular structure of the speckle field, by proper patterning the sample chamber, it is also possible to adjust the average intensity of the field covering an area over the sample or apply an additional intensity gradient, i.e., perform space-time-varying fields. Therefore, dynamic control can be applied to the samples with the important advantages of ST over the typical regular pattern of trapping sites. The requirement to only a few optical components and saving physical space, insensitivity to optical aberrations, use of unfocused light, and no need for sophisticated alignment and adjustment processes, are some of the ST advantages. These benefits make ST a simple, cost-effective, and versatile methodology for applications in various fields, such as drug delivery, lab-on-a-chip, active matter, and in-vivo applications. We believe that the principal idea behind the applications of the optical ST can also be extended to acoustical trapping, which has very recently attracted intensive attention.

### Conflicts of interest

The authors declare no conflicts of interest.

### Funding

This work was partially supported by Photonics, Laser, Advanced Materials and Manufacturing Technologies Development Council of Iran, under Grant No. 97-1-33.
Acknowledgements

The authors would like to thank Saeed Mollaei and Mohammad-Reza Aghdaei for their assistance in performing the experiments, and Bahman Farnudi for linguistic editing of the manuscript.

References

1. Grier, D. G. A revolution in optical manipulation. *Nature* **424**, 810–816 (2003).
2. Hertlein, C., Helden, L., Gambassi, A., Dietrich, S. & Bechinger, C. Direct measurement of critical casimir forces. *Nature* **451**, 172–175 (2008).
3. Munday, J. & Capasso, F. Repulsive casimir and van der waals forces: From measurements to future technologies. *Int. J. Mod. Phys. A* **25**, 2252–2259 (2010).
4. Tien, P. v. Light waves in thin films and integrated optics. *Appl. Opt.* **10**, 2395–2413 (1971).
5. Campbell, G. S. & Norman, J. An introduction to environmental biophysics (Springer Science & Business Media, 2012).
6. Wiersma, D. S. Disordered photonics. *Nat. Photonics* **7**, 188–196 (2013).
7. Rotter, S. & Gigan, S. Light fields in complex media: Mesoscopic scattering meets wave control. *Rev. Mod. Phys.* **89**, 015005 (2017).
8. Jacucci, G., Schertel, L., Zhang, Y., Yang, H. & Vignolini, S. Light management with natural materials: From whiteness to transparency. *Adv. Mater.* **2001215** (2020).
9. Wiersma, D. S. & Cavalieri, S. A temperature-tunable random laser. *Nature* **414**, 708–709 (2001).
10. Redding, B., Liew, S. F., Sarma, R. & Cao, H. Compact spectrometer based on a disordered photonic chip. *Nat. Photonics* **7**, 746 (2013).
11. Eichenfield, M., Michael, C. P., Perahia, R. & Painter, O. Actuation of micro-optomechanical systems via cavity-enhanced optical dipole forces. *Nat. Photonics* **1**, 416 (2007).
12. Grigorenko, A., Roberts, N., Dickinson, M. & Zhang, Y. Nanometric optical tweezers based on nanostructured substrates. *Nat. Photonics* **2**, 365–370 (2008).
13. Shi, J. et al. Acoustic tweezers: patterning cells and microparticles using standing surface acoustic waves (ssaw). *Lab on a Chip* **9**, 2890–2895 (2009).
14. Fan, D., Zhu, F., Cammarata, R. & Chien, C. Electric tweezers. *Nano Today* **6**, 339–354 (2011).
15. Nilsson, J., Evander, M., Hammarström, B. & Laurell, T. Review of cell and particle trapping in microfluidic systems. *Anal. chimica acta* **649**, 141–157 (2009).
16. Yanik, M. F., Rohde, C. B. & Pardo-Martin, C. Technologies for micromanipulating, imaging, and phenotyping small invertebrates and vertebrates. *Annu. review biomedical engineering* **13**, 185–217 (2011).
17. Ghosh, S. & Ghosh, A. Mobile nanotweezers for active colloidal manipulation. *Sci. Robotics* **3**, eaaq0076 (2018).
18. Spyratou, E., Makropoulou, M., Mourelatou, E. & Demetzos, C. Biophotonic techniques for manipulation and characterization of drug delivery nanosystems in cancer therapy. *Cancer Lett.* **327**, 111–122 (2012).
19. Ashkin, A. Acceleration and trapping of particles by radiation pressure. *Phys. Rev. Lett.* **30**, 156 (1970).
20. Verkerk, P. et al. Dynamics and spatial order of cold cesium atoms in a periodic optical potential. *Phys. Rev. Lett.* **68**, 3861 (1992).
21. Haroche, S. Nobel lecture: Controlling photons in a box and exploring the quantum to classical boundary. *Rev. Mod. Phys.* **85**, 1083 (2013).
22. Curtis, J. E., Koss, B. A. & Grier, D. G. Dynamic holographic optical tweezers. *Opt. Commun.* **207**, 169–175 (2002).
23. Gao, D. et al. Optical manipulation from the microscale to the nanoscale: fundamentals, advances and prospects. *Light Sci. & Appl.* **6**, e17039–e17039 (2017).
24. Miyazaki, M. & Hayasaki, Y. Motion control of low-index microspheres in liquid based on optical repulsive force of a focused beam array. *Opt. Lett.* **34**, 821–823 (2009).
25. Peng, F., Yao, B., Yan, S., Zhao, W. & Lei, M. Trapping of low-refractive-index particles with azimuthally polarized beam. *JOSA B* **26**, 2242–2247 (2009).
26. Prentice, P. et al. Manipulation and filtration of low index particles with holographic laguerre-gaussian optical trap arrays. *Opt. Express* **12**, 593–600 (2004).
27. Dasgupta, R., Ahlawat, S., Verma, R. S., Shukla, S. & Gupta, P. K. Optical trapping of spermatozoa using laguerre-gaussian laser modes. *J. biomedical optics* **15**, 065010 (2010).
28. Putterman, S., Evans, P., Vazquez, G. & Weninger, K. Is there a simple theory of sonoluminescence? *Nature* **409**, 782–783 (2001).
29. De Jong, N., Bouakaz, A. & Frinking, P. Basic acoustic properties of microbubbles. *Echocardiography* **19**, 229–240 (2002).
30. Ye, J. Y. et al. Trapping cavitation bubbles with a self-focused laser beam. *Opt. Lett.* **29**, 2136–2138 (2004).
31. Discher, D. E. & Eisenberg, A. Polymer vesicles. *Science* **297**, 967–973 (2002).
32. Mohanty, S., Verma, R. & Gupta, P. Trapping and controlled rotation of low-refractive-index particles using dual line optical tweezers. *Appl. Phys. B* **87**, 211–215 (2007).
33. Tachibana, K., Uchida, T., Ogawa, K., Yamashita, N. & Tamura, K. Induction of cell-membrane porosity by ultrasound. *The Lancet* **353**, 1409 (1999).
34. Zarif, L. Drug delivery by lipid cochleates. In *Methods in enzymology*, vol. 391, 314–329 (Elsevier, 2005).
35. Hansen, P. M., Bhatia, V. K., Harrit, N. & Oddershede, L. Expanding the optical trapping range of gold nanoparticles. *Nano Lett.* **5**, 1937–1942 (2005).
36. Rui, G., Wang, X. & Cui, Y. Manipulation of metallic nanoparticle with evanescent vortex bessel beam. *Opt. Express* **23**, 25707–25716 (2015).
37. Gahagan, K. & Swartzlander, G. Simultaneous trapping of low-index and high-index microparticles observed with an optical-vortex trap. *JOSA B* **16**, 533–537 (1999).
38. Liu, Z. et al. Simultaneous trapping of low-index and high-index microparticles using a single optical fiber bessel beam. *Opt. Lasers Eng.* **131**, 106119 (2020).
39. Bouchaud, J.-P. & Georges, A. Anomalous diffusion in disordered media: statistical mechanisms, models and physical applications. *Phys. Reports* **195**, 127–293 (1990).
40. Goodman, J. W. *Speckle phenomena in optics: theory and applications* (Roberts and Company Publishers, 2007).
41. Volpe, G., Volpe, G. & Gigan, S. Brownian motion in a speckle light field: tunable anomalous diffusion and selective optical manipulation. *Sci. Reports* **4**, 3936 (2014).
42. Shvedov, V. G. et al. Selective trapping of multiple particles by volume speckle field. *Opt. express* **18**, 3137–3142 (2010).
43. Chubynsky, M. V. & Slater, G. W. Diffusing diffusivity: a model for anomalous, yet brownian, diffusion. *Phys. Rev. Lett.* **113**, 098302 (2014).
44. Kotnala, A., Kollipara, P. S. & Zheng, Y. Opto-thermoelectric speckle tweezers. *Nanophotonics* **9**, 927–933 (2020).
45. Nunes, A. S. et al. Ordering of binary colloidal crystals by random potentials. *Soft Matter* **16**, 4267–4273 (2020).
46. Pinçe, E. et al. Disorder-mediated crowd control in an active matter system. *Nat. Commun.* **7**, 1–8 (2016).
47. Recanello, M., Lenzi, E., Martins, A., Li, Q. & Zola, R. Extended adsorbing surface reach and memory effects on the diffusive behavior of particles in confined systems. *Int. J. Heat Mass Transf.* **151**, 119433 (2020).
48. Freund, I. ‘1001’ correlations in random wave fields. *Waves random media* **8**, 119–158 (1998).
49. Berry, M. & Dennis, M. Phase singularities in isotropic random waves. *Proc. Royal Soc. London. Ser. A: Math. Phys. Eng. Sci.* **456**, 2059–2079 (2000).
50. Bender, N., Yilmaz, H., Bromberg, Y. & Cao, H. Customizing speckle intensity statistics. *Optica* **5**, 595–600 (2018).
51. García, L. P., Pérez, J. D., Volpe, G., Arzola, A. V. & Volpe, G. High-performance reconstruction of microscopic force fields from brownian trajectories. *Figshare https://doi.org/10.6084/m9.figshare.7181888* (2018).
52. Shimoni, Y., Nudelman, G., Hayot, F. & Sealfon, S. C. Multi-scale stochastic simulation of diffusion-coupled agents and its application to cell culture simulation. *PloS One* **6** (2011).
53. García, L. P., Pérez, J. D., Volpe, G., Arzola, A. V. & Volpe, G. High-performance reconstruction of microscopic force fields from brownian trajectories. *Nat. Commun.* **9**, 1–9 (2018).
54. Segovia-Gutiérrez, J. P., Escobedo-Sánchez, M. A., Sarmiento-Gómez, E. & Egelhaaf, S. U. Diffusion of anisotropic particles in random energy landscapes-an experimental study. *Front. Phys.* **7**, 224 (2019).
55. Kelly, K. L., Coronado, E., Zhao, L. L. & Schatz, G. C. The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. *The J. Phys. Chem. B* **107**, 668–677, DOI: 10.1021/jp026731y (2003). https://doi.org/10.1021/jp026731y.

56. Jonáš, A. & Zemánek, P. Light at work: The use of optical forces for particle manipulation, sorting, and analysis. *Electrophoresis* **29**, 4813–4851, DOI: 10.1002/elps.200800484 (2008). https://onlinelibrary.wiley.com/doi/pdf/10.1002/elps.200800484.

57. Shvedov, V. G., Hnatovsky, C., Eckerskorn, N., Rode, A. V. & Krolikowski, W. Polarization-sensitive photophoresis. *Appl. Phys. Lett.* **101**, 051106 (2012).

58. Jonáš, A. & Zemanek, P. Light at work: The use of optical forces for particle manipulation, sorting, and analysis. *Electrophoresis* **29**, 4813–4851 (2008).

59. Lehmuskero, A., Johansson, P., Rubinsztein-Dunlop, H., Tong, L. & Kall, M. Laser trapping of colloidal metal nanoparticles. *ACS Nano* **9**, 3453–3469 (2015).