



Determining errors in force measurements for colloids close to contact

Harun Yücel¹

Received: 25 February 2025 / Accepted: 30 May 2025
© The Author(s) 2025

Abstract

Due to diffraction patterns in light microscopy, the overlapping of colloid images introduces minor errors in determining particle positions, which affecting the accuracy of observing interactions between colloids near contact. This study quantitatively investigates the errors arising from the overlapping effect in force measurements between two closely positioned colloids using digital video microscopy (DVM). For this aim, computer simulations were conducted to obtain the positions of two optically trapped colloids interacting based on a force model and particle images were generated as a time series using experimental particle intensity profiles for two different particle sizes. These generated images were analyzed using tracking algorithms from the literature and the interaction forces were extracted and compared with theoretical force values. The results demonstrated that diffraction patterns significantly influence the accuracy of particle position detection and interaction force calculations. Large diffraction patterns and intensity fluctuations in an image introduce significant errors. However, these errors can become significantly smaller in the presence of large forces and may therefore be considered negligible.

Keywords Force measurement · Colloid interactions · Tracking · Simulation

1 Introduction

The measurement of forces between two interacting particles in a colloidal system is essential for understanding micro- and nano-scale physics [1–5]. Over the last several decades, the development of the optical trapping (OT) technique [6–8] combined with digital video microscope (DVM) [9, 10] has made it possible to measure forces at the pico-Newton (pN) level, enabling the investigation of such systems [11–14].

Observing the motion of a colloidal particle suspended in a liquid through video imaging provides understanding how its movement is influenced by its surrounding environment. Particle tracking in images and extracting its motion as a time series are crucial, particularly when colloids approach one another. At close distances, forces such as van der Waals, electrostatic, and hydration interactions significantly influence their behavior. Therefore, accurate measurements of these interactions under near-contact conditions, along with

an understanding of potential errors, are essential for reliable analysis.

Nevertheless, DVM has a limitation in monitoring the positions of particles at close separations [15–18]. The interacting force of two colloids is extracted by analyzing the distance between the centers of particles, based on positions obtained from tracking algorithms applied to particle images. At close particle separations, the position values include errors due to the overlapping effect in the image, leading to inaccuracies in force measurements. A few specialized tracking algorithms that address this issue have been reported in the literature [19–23], alongside conventional tracking algorithms [9, 24]. Similar tracking algorithms are widely applied across a broad range of fields, including motion analysis in computer vision, robotics, and eye tracking for human-computer interaction and medical diagnostics [25, 26].

In an experiment measuring interaction forces with DVM, various errors occur from factors such as position determination during image analysis, system calibration and statistical uncertainties. The overlapping effect is a specific issue encountered during image analysis and contributes to other error types when particles come close to contact. Due to the fact that interaction forces typically occur at close distances, the overlapping effect has a specific importance in such mea-

✉ Harun Yücel
harunyucel@bayburt.edu.tr

¹ Department of Opticianry, Bayburt University, Bayburt 69000, Türkiye

measurements. Thus, this study focuses on the errors caused by the overlapping effect during image analysis.

The question of how much errors in force measurement arise from position errors due to the overlapping effect remains unclear. Is it negligible? [27] This study focuses on determining the absolute force errors in measurements for two colloids close to contact. To achieve this aim, simulations were performed for two interacting colloids in a double optical trap, based on a force model. The simulations generated particle positions and produced images using intensity profiles of the particles for two different particle sizes, which simulating experimental video recordings. This allows quantitatively determining the absolute errors caused by the overlapping effect as the theoretical force is known.

In this study, computer simulations were performed for two optically trapped, interacting colloidal particles. A gravitational-like interaction model was employed to simulate the attractive forces between the particles. The simulations generated time series of particle images, which were analyzed using position tracking algorithms. Based on the extracted positions, interaction forces were calculated and compared with the theoretical force values defined in the simulations. This comparison enabled the evaluation of both absolute and relative errors in the force measurements. The results show that the dominant error arises from image-based position determination, especially due to overlapping diffraction patterns. It was also observed that as the magnitude of the interaction force increases, the relative error decreases.

2 Materials and methods

In this study, simLightForce [28, 29], a simulator for micron-sized particles in an optical force field, has been used to simulate a double optical trap. The simulator employs a geometrical optics approach to calculate the optical forces on a particle and generates particle trajectories for various optical fields. In simLightForce, particles are modeled as spherical to compute the force acting on the particle's center. Assuming spherical symmetry simplifies the calculations. Another key assumption is that the system operates in the far field, where the optical rays are considered to encounter the particles in a parallel manner. A key limitation of the code concerns the size of the particles. For particles with diameters of several microns, the geometric optics approach is appropriate. However, for smaller particles, alternative methods such as electromagnetic or dipole approximations for calculating optical forces are available in the literature [30].

Figure 1b illustrates the refraction of a single ray on a spherical particle. The vertical difference (Δr) between the encounter point of the incident ray and the outgoing point

of the refracted ray on the surface of the particle can be expressed as follows [31, 32].

$$\Delta(r) = \frac{r}{R} \sqrt{1 - \left(\frac{r}{nR}\right)^2} - \frac{r}{nR} \sqrt{1 - \left(\frac{r}{R}\right)^2} \quad (1)$$

Here, r represents the radial distance from the center and R is the radius. n represents the ratio of n_p/n_m , where $n_p = 1.59$ denotes the refractive index of the particles, and $n_m = 1.33$ denotes the refractive index of the medium.

The optical field is represented as a grayscale pattern $I(M \times N)$, where $M = 250$ and $N = 500$ denote the pixel dimensions representing the number of rows and columns, respectively. The optical force ($\vec{F}^{opt.}$) acting on the particle can be expressed as an integral over the particle area.

$$\vec{F}^{opt.} = \kappa \iint I(M \times N) \Delta(r) dA \quad (2)$$

The κ parameter is a conversion factor that relates the grayscale intensity of the pattern (I) to the light power. The optical field (I) follows a double 2D Gaussian distribution with a standard deviation of 0.5 as shown in Fig. 1c. This is a way to create a double optical trap.

To simulate the interaction between two colloidal particles, the simulator has been adapted to include Newtonian gravitational interaction as an interaction model in this work. The primary goal is to assess systematic errors in position-based force measurements, where the position errors themselves are independent of the specific interaction model used. Although Newtonian gravitational interaction is not a typical force in colloidal systems, it was chosen here for its computational simplicity and to mimic a weak, long-range interaction. More importantly, it follows an inverse-square dependence on interparticle distance commonly encountered in physical interactions, providing a smooth and well-behaved force profile. The interaction is extremely weak, making it challenging to detect in a double optical trap experiment. Therefore, the gravitational constant (G) was assigned an unrealistic value of $1 \text{ fN}\mu\text{m}^2/\text{pg}^2$, whereas its real value is $6.674 \times 10^{-14} \text{ fN}\mu\text{m}^2/\text{pg}^2$.

$$\vec{F}_{12}^{int.} = -\vec{F}_{21}^{int.} = G \frac{m_1 m_2}{|\vec{d}_{12}|^2} \hat{d}_{12} \quad (3)$$

As described in Fig. 1a, d is the distance from the center of one particle to the center of the other particle. The particle masses, m_1 and m_2 , were assumed to be equal and were calculated based on a density of 1.85 g/cm^3 [33]. Their values are 163.8119 pg and 7.7493 pg for silica particles with diameters of $5.53 \mu\text{m}$ and $2.00 \mu\text{m}$, respectively. In simulations of a two-particle system, the trajectories of the particles were generated by numerically solving the Langevin equa-

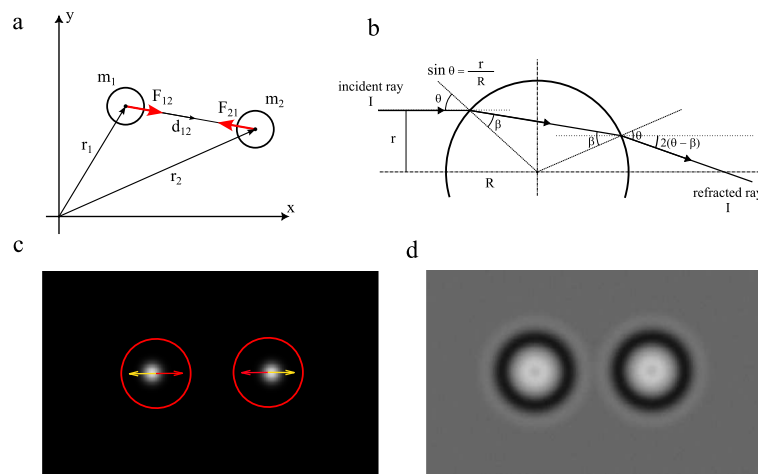


Fig. 1 (a) describes the coordinates of a two-body interaction system. (b) defines geometrically the refraction of a single ray through a particle to calculate the momentum transfer of light to the particle. (c) illustrates the double 2D Gaussian pattern that creates optical trap centers. Red circles represent particles, while red arrows indicate the rescaled inter-

action force vectors on the x -dimension between colloids. Similarly, yellow arrows corresponds to the optical restoring force vectors acting on the particles along the x -dimensions. (d) depicts the particle images corresponding to the positions illustrated in (c)

tion for the x and y components of the position, with optical and interaction force terms added, as shown below [34].

$$\begin{aligned} x(n) &= x(n-1) + [F_x^{int.}(n-1) + F_x^{opt.}(n-1)] \frac{\Delta t}{\gamma} + \sqrt{2D\Delta t}w(n) \\ y(n) &= y(n-1) + [F_y^{int.}(n-1) + F_y^{opt.}(n-1)] \frac{\Delta t}{\gamma} + \sqrt{2D\Delta t}w(n) \end{aligned} \quad (4)$$

Here, n refers to the index of the discretized time steps in the simulation, $\gamma = 6\pi\eta R$ and $D = K_b T / \gamma$. R is the particle radius, $\eta = 1 \text{ fNs}/\mu\text{m}^2$ is the viscosity of the liquid, $T = 300 \text{ K}$ is the temperature, and $K_b = 0.0138 \text{ fN}\mu\text{mK}^{-1}$ is the Boltzmann constant. $w(n)$ represents white noise with a zero (0) mean and unit (1) variance for the x and y components separately.

After generating the trajectory, the particle images corresponding to the positions were produced as a time series. For image generation, the experimental particle intensity profile described in [20] was used. In the simulation of $5.53 \mu\text{m}$ diameter particles, the intensity profile was obtained by interpolation from an experimental particle image shown in Fig. 1d. For $2.00 \mu\text{m}$ diameter particles, the intensity profile was obtained by fitting a Gaussian-Bessel function to an experimental particle image shown in Fig. 4c.

To generate multi-particle images, a linear combination of individual particle profiles was used in the following form:

$$I_{\text{image}} = I_{1p} + I_{2p} + I_0 + I_{\text{noise}} \quad (5)$$

Here, I_{1p} and I_{2p} are the intensity distributions of the first and second individual particles, respectively. I_0 represents the background intensity, and I_{noise} denotes the simulated noise in the image.

For noise generation in the images, random fluctuations which an amplitude of 5 units in grayscale was added. Example of generated particle images are shown in Fig. 1d for $5.53 \mu\text{m}$ diameter particles and in Fig. 4c for $2.00 \mu\text{m}$ diameter particles. In these simulations, one pixel corresponds to $0.08 \mu\text{m}$.

3 Results and discussions

As understood from the previous section, no trap stiffness parameter is used to simulate particle trajectories. Instead, the optical force is calculated from the 2D double Gaussian intensity distribution pattern. Therefore, it is necessary to calibrate the optical traps. In this study, the trap stiffness values were obtained using a method similar to the drag force method [12, 14, 35]. However, rather than employing a drag force, a constant external force applied along the x direction was considered. Particle trajectories were generated under the influence of the constant external force exerted on the particle.

Figure 2 presents the calibration results for $5.53 \mu\text{m}$ diameter particles, including particle trajectories. During calibration, a linear fit was performed for the average displacement (Δx) of the particle positions versus different external force values. The obtained stiffness parameter is $k_x^{(1)} = 2.6394 \pm 0.03 \text{ pN}/\mu\text{m}$. When the calibration was repeated using statistical methods such as potential analysis and the mean square displacement (MSD) method, the trap stiffness parameters were found to be $k_x^{(2)} = 2.5905 \pm 0.072 \text{ pN}/\mu\text{m}$ and $k_x^{(3)} = 2.4029 \pm 0.001 \text{ pN}/\mu\text{m}$, respectively. As shown in Fig. 2b, the stiffness parameters exhibit no significant differences. In the simulations of $2.00 \mu\text{m}$

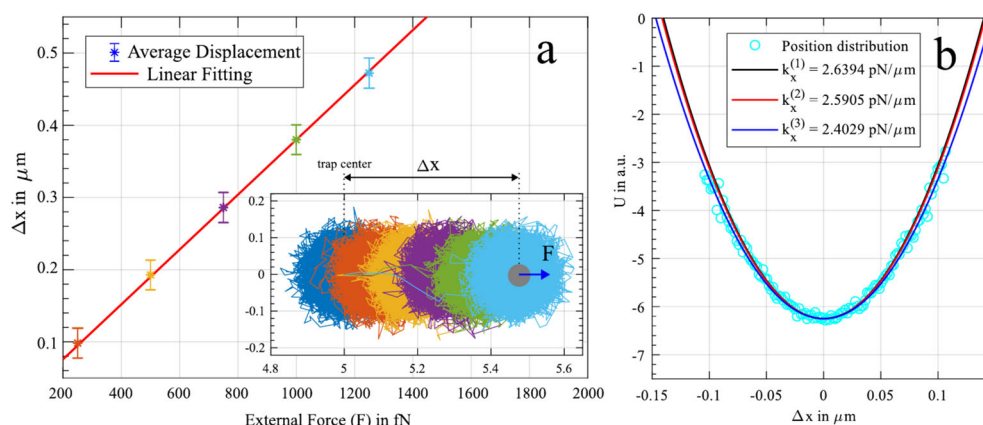


Fig. 2 The calibration results for $5.53 \mu\text{m}$ diameter particles are presented as follows: **(a)** shows the fitting results for the average displacement corresponding to the external force exerted on the particle. The inset in **(a)** displays particle trajectories under different external force values. **(b)** presents the position distribution in the

absence of external force, along with theoretical calculation results $((1/2)k_x \Delta x^2 - 6.2487)$ for various trap stiffness values. In the graph, the black line represents the external force method, the red line denotes the potential analysis method, and the blue line corresponds to the MSD method calibration

diameter particles, the stiffness parameter was found to be $492.5472 \pm 6.33 \text{ fN}/\mu\text{m}$ under the influence of a constant external force exerted on the particle.

For the interaction simulation, the distance between the 2D double Gaussian patterns (trap centers) was varied from 6.0 to $12.0 \mu\text{m}$ in increments of $0.2 \mu\text{m}$, and the trajectories of the particles were recorded for 100 s at a frame rate of 100 frames per second. The recorded images were subsequently analyzed using radial symmetry centers method (RMS) [24] and partial radial symmetry centers method (pRMS) [20] and the average equilibrium positions were determined for each center-to-center distance.

The RMS method uses the gradient vectors of the particle intensity distribution in the image. These gradient vectors orient toward the brightest point in the image, which is considered as the particle position. The pRMS method, a refined version of RMS, utilizes the gradient vectors from the unaffected regions of the particle intensity distribution in determining the particle position, excluding contributions from neighboring particles.

To simplify the analysis, only the horizontal components of forces and distances were considered. The interaction force acting on one colloid due to the other was calculated based on the displacement (Δx) of the average equilibrium position from the trap center. At this point, the colloid is in equilibrium as the optical restoring force is balanced by the interaction force acting on the opposite direction ($F_x^{\text{int.}} = -k_x \Delta x$ and see Fig. 1c). The resulting force values are presented in Fig. 3. The graph indicates no significant difference between the tracking methods at larger center-to-center distances. However, minor inconsistency appear when the particles are in close contact. While the RSM method

introduces small errors, the pRSM method, a refined version of RSM, demonstrates improved accuracy with respect to the theoretical force values.

The simulations performed above were repeated for $2 \mu\text{m}$ diameter particles having a different diffraction pattern. In image generation of $2 \mu\text{m}$ diameter particle, a Gaussian-Bessel function, $I_p = (A - I_0) \exp(-K^2 r^2) J_0(r/L)$, was used with parameters $A = 180$, $I_0 = 60$, $K = 5$ and $L = 1/30$. The diffraction pattern is similar to the one commonly observed in microscopy and the intensity distribution extends over a long distance from the particle surface. Therefore, the determining position when the particles are close to each other is a challenge.

In the simulations of $2 \mu\text{m}$ diameter particle, the optical stiffness parameter (k_x) were obtained as $492.5472 \pm 6.33 \text{ fN}/\mu\text{m}$. The distance between the optical trap centers was varied from 2.0 to $8.0 \mu\text{m}$ with a step size of $0.2 \mu\text{m}$ and the particle trajectories were recorded for 100 s at a frame rate of 100 frames per second. The recorded images were subsequently analyzed using only the RMS method as the particle intensity distribution was inconsistent for applying the pRMS method. The pRMS method determines the particle position by using the unaffected parts of the particle intensity distribution from those of neighboring particles. Due to the small central brightness region of the intensity distribution, the pRMS method has limitations for $2 \mu\text{m}$ diameter particles. The simulation, conducted for particles with a diameter of $2 \mu\text{m}$, was repeated with different values of the interaction model constant $G = 5$ to increase the force magnitude.

Using a similar analysis to that conducted for $5.53 \mu\text{m}$ diameter particles, the interaction force results for $2 \mu\text{m}$ diameter particles are presented in Fig. 4a and b. Panel

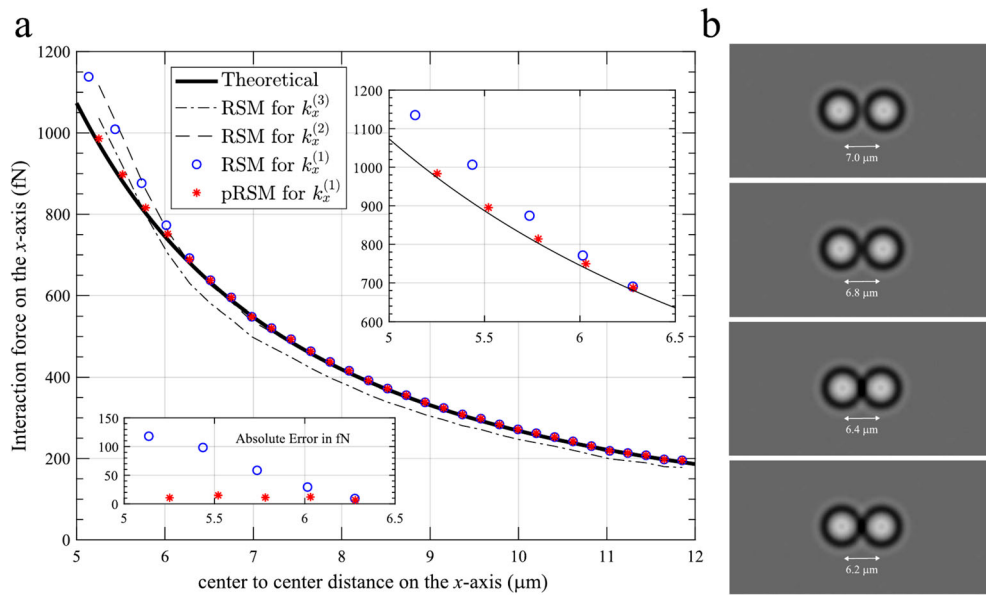


Fig. 3 (a) illustrates the force measurement results, where the blue circles correspond to analyses obtained using the radial symmetry method (RSM) [24], and the red points represent those obtained using the partial radial symmetry method (pRSM) [20]. The black line represents the theoretical prediction of the interaction model, while the dashed lines correspond to the calculated force values using trap stiffness

obtained from potential analysis and the mean square displacement (MSD) method. In Figure (a), the upper inset provides a magnified view of a specific region of the graph, while the lower inset depicts the absolute errors as a function of distance, showing a maximum error of 120 fN at the contact condition. (b) displays particle images used during the simulation at various center-to-center distances

(a) presents the results for the simulation conducted with $G = 1$, and panel (b) shows the results for $G = 5$ in the interaction model. As can be seen from the figure, inconsistencies between the measured and theoretical values begin to appear when the distance is smaller than $5 \mu\text{m}$, which corresponds to a value larger than the particle diameter. Additionally, the incoherences between the measured and theoretical values are more pronounced compared to those observed for $5.53 \mu\text{m}$ diameter particles (Fig. 4a). It is understood that the reason for these results lies in the diffraction patterns of the particles. The diffraction pattern of $5.53 \mu\text{m}$ diameter particles exhibits a short-range effect, while that of the $2.00 \mu\text{m}$ diameter particles displays a long-range effect.

The error in the measured force in such an experiment arises from a combination of several sources, including position determination, calibration, and thermal fluctuations [9]. Specifically, the uncertainty in the force $F = k_x x$ can be expressed using standard error propagation as

$$\Delta F = \sqrt{(x \Delta k_x)^2 + (k_x \Delta x)^2} \quad (6)$$

where Δk_x is the uncertainty in the trap stiffness determined through calibration, and Δx is the uncertainty in the particle's displacement from the trap center. The total positional uncertainty Δx includes contributions from both thermal

fluctuations and image-based position determination errors. These sources are statistically independent and can be combined in quadrature:

$$\Delta x = \sqrt{\Delta x_{\text{thermal}}^2 + \Delta x_{\text{image}}^2}$$

Here, $\Delta x_{\text{thermal}} = \sqrt{k_B T / k_x}$ (which evaluates to 0.0917, corresponding to $\Delta F_{\text{thermal}} = k_x \Delta x_{\text{thermal}} = 45.16$ fN) represents the positional fluctuations due to Brownian motion within the optical trap [36], and Δx_{image} accounts for errors introduced during position determination, such as pixelation and camera noise.

In time series analysis, block averaging is a technique used to reduce noise and estimate the uncertainty of measured quantities [37]. The time series of position measurements is divided into N consecutive, non-overlapping blocks. By averaging the displacements within each block, the random fluctuations due to measurement noise are smoothed out. The standard deviation σ of the block means reflects the variability among these block averages, and the standard error of the mean (SEM) is given by

$$\Delta x_{\text{image}} = \frac{\sigma}{\sqrt{N}}$$

where $N = 20$ is the number of blocks (for 100 seconds of data with 5-second blocks), and σ is the standard devi-

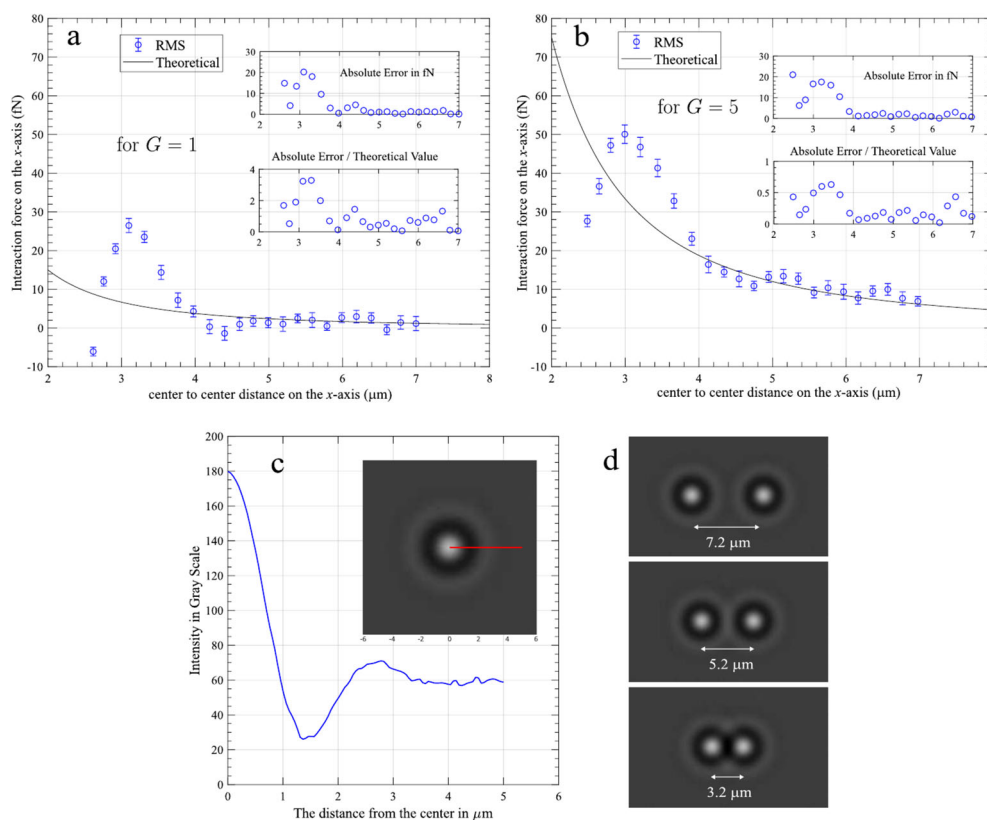


Fig. 4 (a) illustrates the force measurement results for $2\ \mu\text{m}$ diameter particles. The blue circles correspond to analyses obtained using the radial symmetry method (RSM) [24], while the black line represents the theoretical calculation of the interaction model (for $G = 1$). The lower inset in (a) shows the absolute errors as a function of distance, showing a maximum error of $50\ \text{fN}$ at the contact condition. (b) illustrates

the force measurement results from the repeated simulations shown in (a) for $G = 5$. (c) presents the intensity profile of the $2\ \mu\text{m}$ diameter particles, corresponding to the red line drawn across the particle image displayed in the upper inset. (d) displays particle images used during the simulation at various center-to-center distances, where the overlapping effect is more apparent compared to $5.53\ \mu\text{m}$ diameter particles

ation of the block-averaged positions. This SEM quantifies the uncertainty in position determination caused by image-based noise, and is taken as Δx_{image} in the force uncertainty calculation.

This uncertainty is represented as error bars in Fig. 4a and b by applying (6). These error bars reflect only the uncertainty arising from the RSM algorithm, where image noise is the dominant factor. The algorithm cannot eliminate the overlapping effects of the diffraction pattern, which contributes to the overall measurement error.

Therefore, the differences between the measured and theoretical values (i.e., the absolute errors) shown in the graphs can be attributed primarily to the contribution of diffraction pattern overlap within the total uncertainty. To better understand this behavior, the relative error is calculated as

$$E_{\text{relative}} = \frac{|F_{\text{meas}} - F_{\text{theo}}|}{F_{\text{theo}}}$$

where F_{meas} and F_{theo} are the measured and theoretical forces, respectively. As shown in Fig. 4a and b, the relative error is approximately 3.30 at a $3\ \mu\text{m}$ center-to-center dis-

tance for the simulation with $G = 1$, but decreases to 0.50 at the same distance for $G = 5$. This indicates that as the force magnitude increases, the relative error decreases, suggesting that higher forces lead to more accurate measurements and reduce the impact of overlapping diffraction patterns. Therefore, the reliability of the force measurements improves with increasing force values.

The maximum error in the interaction force measurement is approximately $120\ \text{fN}$ ($0.12\ \text{pN}$) for $5.53\ \mu\text{m}$ diameter particles and $50\ \text{fN}$ for $2.00\ \mu\text{m}$ diameter particles under close-contact conditions for the RSM method. When considering the relative error between the measurement absolute error and the theoretical force values, the relative error are approximately 0.11 for $5.53\ \mu\text{m}$ particles and 3.33 for $2.00\ \mu\text{m}$ particles. For $5.53\ \mu\text{m}$ diameter particles, the ratio is small because the diffraction pattern of these particles has a limited effect on the intensity distribution of neighboring particles (see Fig. 3b). This minimizes the overlapping effect and reduces errors in the interaction force measurements. For $2.00\ \mu\text{m}$ diameter particles, the ratio is larger due to the more extensive diffraction pattern, which significantly affects the

intensity distribution of neighboring particles (see Fig. 4c and d). This increased overlapping effect leads to greater errors in the interaction force measurements.

Calibration errors typically arise from uncertainties in pixel size, optical distortions, and instrument stability. In well-calibrated systems, these errors are generally on the order of tens of nanometers in position, which corresponds to a few femto-Newtons in force estimation depending on the force gradient. Thermal noise, caused by the Brownian motion of colloidal particles, introduces fluctuations that depend on particle size, temperature, and measurement timescale. For particles of the sizes considered here, thermal force fluctuations are typically on the order of a few tens of femto-Newtons. Therefore, the systematic errors due to diffraction pattern overlap reported in this work, reaching up to 120 fN, can be comparable to or even exceed other common sources of error in digital video microscopy, especially when measuring weak forces. This underscores the importance of accounting for such systematic effects to improve the accuracy of force measurements.

The overlapping of particle images is caused by the diffraction patterns of the particles. Larger diffraction patterns result in greater errors in position detection. Furthermore, fluctuations in the intensity of the diffraction pattern can substantially distort the intensity distribution of nearby particles, amplifying the error magnitude. Adjusting the intensity of the particle diffraction pattern during experiments may help reduce these errors. In such cases, the overlapping effect may become negligible during analysis (see Fig. 3a).

The overlapping effect distorts the information of the particle position. Especially when the interaction between the particles depends on the distance between them, this distortion can lead to inaccuracies in force measurements and interaction analysis. The overlapping of diffraction patterns not only affects the precision of positional data but also introduces systematic errors that become more pronounced as the particles approach to contact (see Fig. 4a). Therefore, minimizing the overlapping effect is crucial for accurate characterization of particle interactions, particularly in systems where precise distance measurements are critical.

To mitigate errors arising from overlapping diffraction patterns, several experimental strategies can be employed. Using high numerical aperture objectives reduces the size of diffraction-limited spots and improves lateral resolution. Additionally, adjusting the particles along the optical (z -) axis may slightly defocus the image, but it can also decrease the spatial extent of the diffraction pattern. Illumination with partially coherent or structured light may help suppress interference effects and enhance image clarity. Furthermore, advanced imaging techniques such as digital holography or deconvolution microscopy can improve positional accuracy by more precisely reconstructing particle locations.

4 Conclusion

This study investigated the errors occurring from the overlapping effect in force measurements between two colloidal particles under close-contact conditions. Using computer simulations, particle trajectories were generated and corresponding particle images were analyzed using established tracking algorithms. The results demonstrated that diffraction patterns significantly influence the accuracy of particle position detection and interaction force calculations. Larger diffraction patterns and their intensity fluctuations introduces greater errors.

The overlapping effect causes systematic errors in determining particle positions. The magnitude of these errors depends on the characteristics of the diffraction patterns in the image. Large diffraction patterns introduce significant errors in observing any interaction forces. Therefore, the error magnitude is independent of the interaction model. When the magnitude of the interaction forces is significantly larger than the magnitude of the overlapping errors, these errors can be considered negligible (see Figs. 3a and 4b, where one can extract the gravitational constant G). However, when the interaction forces are small compared to the overlapping errors (i.e., high relative error), the measured interaction forces contain significant systematic errors (see Fig. 4a), making it challenging to extract the gravitational constant G . This difficulty can severely limit the accuracy of interaction analysis, particularly when the underlying interaction model is not known.

To estimate image-based uncertainty in particle position, the block averaging technique was employed. This approach allowed for a quantification of the standard error arising from image analysis noise. Our results show that errors caused by diffraction pattern overlap significantly impair force accuracy, particularly at short interparticle distances. Furthermore, the relative error between measured and theoretical force values decreases as the interaction strength increases, indicating that stronger forces yield more reliable measurements.

The findings highlight the importance of minimizing the overlapping effect to improve the accuracy of measurements, especially in systems where precise interparticle distances are critical for interaction analyses. Refining tracking algorithms, reducing diffraction pattern intensities or developing alternative imaging techniques could provide pathways to reduce these errors. This work provides views on the challenges and potential solutions in accurately measuring interaction forces between closely spaced colloids in optical trapping experiments.

Author Contributions The entire process of the article was carried out by HY.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This work was supported by open access funding from TÜBİTAK.

Data Availability The raw data supporting the conclusions of this article will be made available by the author on request.

Declarations

Conflicts of Interest The author declares no conflicts of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. L. Belloni, Colloidal interactions. *J. Phys. Condens. Matter* **12**(46), 549 (2000). <https://doi.org/10.1088/0953-8984/12/46/201>
2. P.L. Biancaniello, J.C. Crocker, Line optical tweezers instrument for measuring nanoscale interactions and kinetics. *Rev. Sci. Instrum.* **77**(11), 113702 (2006). <https://doi.org/10.1063/1.2387893>
3. M. Polin, D.G. Grier, Y. Han, Colloidal electrostatic interactions near a conducting surface. *Phys. Rev. E* **76**(4), 041406 (2007). <https://doi.org/10.1103/PhysRevE.76.041406>. Publisher: American Physical Society
4. O. Akhavan, M. Saadati, M. Jannesari, Graphene Jet Nanomotors in Remote Controllable Self-Propulsion Swimmers in Pure Water. *Nano Lett.* **16**(9), 5619–5630 (2016). <https://doi.org/10.1021/acs.nanolett.6b02175>. Publisher: American Chemical Society
5. S. Wang, R. Walker-Gibbons, B. Watkins, M. Flynn, M. Krishnan, A charge-dependent long-ranged force drives tailored assembly of matter in solution. *Nat. Nanotechnol.* **19**(4), 485–493 (2024). <https://doi.org/10.1038/s41565-024-01621-5>
6. A. Ashkin, Acceleration and Trapping of Particles by Radiation Pressure. *Phys. Rev. Lett.* **24**(4), 156–159 (1970). <https://doi.org/10.1103/PhysRevLett.24.156>. Publisher: American Physical Society
7. A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, S. Chu, Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* **11**(5), 288–290 (1986). <https://doi.org/10.1364/OL.11.000288>. Publisher: Optica Publishing Group
8. L.P. Ghislain, W.W. Webb, Scanning-force microscope based on an optical trap. *Opt. Lett.* **18**(19), 1678–1680 (1993). <https://doi.org/10.1364/OL.18.001678>. Publisher: Optica Publishing Group
9. J.C. Crocker, D.G. Grier, Methods of Digital Video Microscopy for Colloidal Studies. *J. Colloid Interface Sci.* **179**(1), 298–310 (1996). <https://doi.org/10.1006/jcis.1996.0217>
10. D.G. Grier, A revolution in optical manipulation. *Nature* **424**(6950), 810–816 (2003). <https://doi.org/10.1038/nature01935>. Publisher: Nature Publishing Group
11. E.-L. Florin, A. Pralle, J.K. Heinrich Hörber, E.H.K. Stelzer, Photonic Force Microscope Based on Optical Tweezers and Two-Photon Excitation for Biological Applications. *J. Struct. Biol.* **119**(2), 202–211 (1997). <https://doi.org/10.1006/jsbi.1997.3880>
12. P.H. Jones, O.M. Maragó, G. Volpe, Optical Tweezers: Principles and Applications. Cambridge University Press, Cambridge (2015). <https://doi.org/10.1017/CBO9781107279711>
13. G. Pesce, G. Volpe, O.M. Maragó, P.H. Jones, S. Gigan, A. Sasso, G. Volpe, Step-by-step guide to the realization of advanced optical tweezers. *JOSA B*. **32**(5), 84–98 (2015). <https://doi.org/10.1364/JOSAB.32.000B84>. Publisher: Optica Publishing Group
14. J. Gieseler, J.R. Gomez-Solano, A. Magazzù, I. Pérez Castillo, L. Pérez García, M. Gironella-Torrent, X. Viader-Godoy, F. Ritort, G. Pesce, A.V. Arzola, K. Volke-Sepúlveda, G. Volpe, Optical tweezers — from calibration to applications: a tutorial. *Adv. Opt. Photon.* **13**(1), 74 (2021). <https://doi.org/10.1364/AOP.394888>
15. J. Baumgartl, C. Bechinger, On the limits of digital video microscopy. *Europhys. Lett.* **71**(3), 487 (2005). <https://doi.org/10.1209/epl/i2005-10107-2>. Publisher: IOP Publishing
16. J. Baumgartl, J.L. Arauz-Lara, C. Bechinger, Like-charge attraction in confinement: myth or truth? *Soft Matter*. **2**(8), 631–635 (2006). <https://doi.org/10.1039/B603052A>. Publisher: The Royal Society of Chemistry
17. M. Polin, Y. Roichman, D.G. Grier, Autocalibrated colloidal interaction measurements with extended optical traps. *Phys. Rev. E* **77**(5), 051401 (2008). <https://doi.org/10.1103/PhysRevE.77.051401>. Publisher: American Physical Society
18. M. Gyger, F. Rückerl, J.A. Käs, J. Ruiz-García, Errors in two particle tracking at close distances. *J. Colloid Interface Sci.* **326**(2), 382–386 (2008). <https://doi.org/10.1016/j.jcis.2008.06.046>
19. C. Zhang, G. Brügger, F. Scheffold, Tracking of colloids close to contact. *Opt. Express* **23**(17), 22579–22586 (2015). <https://doi.org/10.1364/OE.23.022579>. Publisher: Optica Publishing Group
20. H. Yücel, N.T. Okumuşoğlu, A new tracking algorithm for multiple colloidal particles close to contact. *J. Phys. Condens. Matter* **29**(46), 465101 (2017). <https://doi.org/10.1088/1361-648X/aa908e>. Publisher: IOP Publishing
21. C. Zhang, J. Muñetón Díaz, A. Muster, D.R. Abujetas, L.S. Froufe-Pérez, F. Scheffold, Determining intrinsic potentials and validating optical binding forces between colloidal particles using optical tweezers. *Nat. Commun.* **15**(1), 1020 (2024). <https://doi.org/10.1038/s41467-024-45162-w>. Publisher: Nature Publishing Group
22. A.D. Kashkanova, A.B. Shkarin, R.G. Mahmoodabadi, M. Blessing, Y. Tuna, A. Gemeinhardt, V. Sandoghdar, Precision single-particle localization using radial variance transform. *Opt. Express* **29**(7), 11070–11083 (2021). <https://doi.org/10.1364/OE.420670>. Publisher: Optica Publishing Group
23. S. Helgadottir, A. Argun, G. Volpe, Digital video microscopy enhanced by deep learning. *Optica* **6**(4), 506–513 (2019). <https://doi.org/10.1364/OPTICA.6.000506>. Publisher: Optica Publishing Group
24. R. Parthasarathy, Rapid, accurate particle tracking by calculation of radial symmetry centers. *Nat. Methods* **9**(7), 724–726 (2012). <https://doi.org/10.1038/nmeth.2071>. Publisher: Nature Publishing Group
25. A. Yilmaz, O. Javed, M. Shah, Object tracking: A survey. *ACM Comput. Surv.* **38**(4), 13 (2006). <https://doi.org/10.1145/1177352.1177355>
26. A.B. Naeini, A.G. Mahdipour, R. Dorri, Using Eye Tracking to Measure Overall Usability of Online Grocery Shopping Websites. *Int. J. Mob. Comput. Multimed. Commun. (IJMCMC)* **14**(1), 1–24 (2023). <https://doi.org/10.4018/IJMCMC.326129>. Publisher: IGI Global Scientific Publishing
27. H.M. Lee, Y.W. Kim, E.M. Go, C. Revadekar, K.H. Choi, Y. Cho, S.K. Kwak, B.J. Park, Direct measurements of the colloidal Debye

- force. *Nat. Commun.* **14**(1), 3838 (2023). <https://doi.org/10.1038/s41467-023-39561-8>
28. H. Yücel, A simulation study on colloid diffusion under time-varying optical potentials. *J. Appl. Phys.* **134**(1), 013101 (2023). <https://doi.org/10.1063/5.0154604>
 29. H. Yücel, *simLightForce*. GitHub. (2023). <https://github.com/yucelharun/simLightForce.git>. Accessed 15 April 2023
 30. G. Pesce, P.H. Jones, O.M. Maragò, G. Volpe, Optical tweezers: theory and practice. *Eur. Phys. J. Plus* **135**(12), 949 (2020). <https://doi.org/10.1140/epjp/s13360-020-00843-5>
 31. E. Sarmiento-Gómez, J.A. Rivera-Morán, J.L. Arauz-Lara, Single particle states of colloidal particles in 2D periodic potentials. *Soft Matter* **14**(19), 3684–3688 (2018). <https://doi.org/10.1039/C8SM00326B>. Accessed 11 Nov 2019
 32. A. Callegari, M. Mijalkov, A.B. Gököz, G. Volpe, Computational toolbox for optical tweezers in geometrical optics. *J. Opt. Soc. Am. B* **32**(5), 11 (2015). <https://doi.org/10.1364/JOSAB.32.000B11>
 33. GmbH: Micro particle properties. (2023). <https://microparticles.de/en/properties>. Accessed 06 April 2023
 34. G. Volpe, G. Volpe, Simulation of a Brownian particle in an optical trap. *Am. J. Phys.* **81**(3), 224–230 (2013). <https://doi.org/10.1119/1.4772632>
 35. P. Wu, R. Huang, C. Tischer, A. Jonas, E.-L. Florin, Direct Measurement of the Nonconservative Force Field Generated by Optical Tweezers. *Phys. Rev. Lett.* **103**(10), 108101 (2009). <https://doi.org/10.1103/PhysRevLett.103.108101>. Publisher: American Physical Society
 36. G.M. Gibson, J. Leach, S. Keen, A.J. Wright, M.J. Padgett, Measuring the accuracy of particle position and force in optical tweezers using high-speed video microscopy. *Opt. Express* **16**(19), 14561–14570 (2008). <https://doi.org/10.1364/OE.16.014561>. Publisher: Optica Publishing Group
 37. H. Flyvbjerg, H.G. Petersen, Error estimates on averages of correlated data. *J. Chem. Phys.* **91**(1), 461–466 (1989). <https://doi.org/10.1063/1.457480>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.