Study of the stability of optical tweezers

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Abstract: The aim of this project is twofold: to analyze the stability of optical tweezers and to implement a new scheme to generate them. The initial analysis is done by comparing the two most common multiple object manipulation techniques, which are the Time-Sharing Optical Tweezers (TSOT) and Holographic Optical Tweezers (HOT). To achieve this, a trap calibration framework is designed using a high-speed camera that allows to analyze particle dynamics with high temporal and spatial resolution. Once the advantages and disadvantages of each method are known, a new scheme is proposed and implemented using elements of the two most used techniques combined. The combination results in simultaneously generating two types of traps, having the speed of TSOT with the stability of HOT. This allows to generate more stable traps than with TSOT, while being compatible with force measurement techniques.

I. INTRODUCTION

Nowadays, optical tweezers are a widely applied technology in the fields of cell biology and physics of out-of-equilibrium systems.[1] That is because they allow manipulation of microscopic objects with nanometric precision by using a highly focused laser beam without damaging the sample. Their most well-known applications are: the study of single molecules such as biopolymers (DNA and RNA) and the study of molecular motors.[2]

This optical micro-manipulation technique was discovered by A. Ashkin, whose studies conducted in 1986 and 1987 demonstrated that a laser beam, under certain conditions, can be used for trapping and manipulating living microorganisms.[3][4]

The optical tweezers interact with the object by applying a net force on it, causing the object to return to the center of the trap if it deviates from the equilibrium due to its Brownian motion or external forces.[1] This net force can be approximated by Hooke's law as follows:[5]

$$F = -kx, \tag{1}$$

Where F is the trapping force, k is the trap stiffness and x is the distance of the trapped particle from the position of equilibrium.

To measure the trapping force, it is necessary to calibrate the optical trap first. The calibration is done by measuring the trap stiffness, which can be obtained by analyzing the thermal fluctuation of the trapped object. A higher stiffness will confine the trapped object in a smaller region of space, from which a trap stiffness value can be associated.

Therefore, trap calibration relies on a precise measurement of the object position, and for that, the two most common methods are the following: laser-based techniques, where the forward scattered light is captured by an additional detection system, providing a voltage proportional to the object position, and video-based techniques, where the position is obtained computationally from the experimental images, which is the one implemented in this work.

Once the stiffness is known, external forces applied on the trapped object are measured from its movement towards the new equilibrium position.[6]

When it comes to generating multiple optical traps, there are two techniques: time-sharing and holographic optical tweezers.

Time-Sharing Optical Tweezers (TSOT) is a technique based on the generation of multiple optical traps from a single laser beam by using a pair of acousto-optical deflectors (AOD). The AODs generate multiple traps by rapidly and periodically deflecting the angle of the beam to the different particle traps positions.[7,8] From the point of view of each particle, the laser is blinking. If the switching frequency is faster than the response time of the trapped object, each individual target averages the intensity fluctuation and responds as being trapped with a continuous laser.

By using this technique, all captured information related to each trapped object is distinguishable for laser-based force detection methods. This is achieved with proper timing and thanks to the fast response of AODs, real-time subject manipulation and feedback control are enabled.

However, this technique also has drawbacks. Since the trap is not static, the object drifts away from its trapped position while the laser is not pointing at it. This phenomenon limits the accuracy of the measurements and becomes more severe as the number of trapped objects increases. This means that the more objects trapped simultaneously, the longer it takes for the laser to visit again the same position.

Holographic Optical Tweezers (HOT) is a technique that uses spatial light modulators (SLM) to shape the waveform of the incoming beam of light with the aim of creating multiple optical traps. This technique generates permanent traps, which means there is no fluctuation in the position of the trapped subjects.

There is, however, a major flaw in this technique. HOTs are not compatible with force laser detection methods due to the traps being generated simultaneously. The detection system always collects light scattered by all particles, making them indistinguishable.

In order to study the stability of the traps in this work, a stiffness calibration method was implemented using a high-speed camera, which allows analyzing the fluctuations and dynamics of the trapped particles with high resolution for both approaches: time-sharing and holography. In this case, the two used techniques were implemented using the same type of AODs device. Lastly, using the advantages of HOTs and TSOTs through a technique developed by BIOPT called Acousto-Holographic Optical Tweezers (AHOT), a two-trap-based scheme is proposed that improves the stability of the traps concerning time-sharing.

II. EXPERIMENTAL PROCEDURE

All the experiments were carried out using a custom-built optical trapping setup from the BIOPT Lab. The optical setup was built around an inverted Nikon TE-2000E microscope and was equipped with a 5W (CW continuous wave) laser λ=1064 nm (IPG YLM-5-1064-LP). The laser was then expanded, redirected and focused toward the high NA microscope objective (NIKON 60X NA1.2, water immersion). To modulate the laser beam, the setup used two orthogonally arranged AODs capable of deflecting the laser at 150 kHz.

To implement camera-based power spectrum analysis, a high-speed camera (Optronis Cyclone 2-2000) was incorporated, allowing to record videos at 2500 fps full-frame and over 15000fps at 256x128 region of interest (ROI).

The samples used throughout the experiments were solutions of 3 μm polystyrene micro-spheres in an aqueous medium, around 70 μl of the solution was deposited in a custom-made microchamber using Scotch tape as a spacer.

A. Particle position tracking and data processing software

Each stiffness calibration experiment consisted of generating the desired trap configuration, trapping the particles, and recording a high-speed video of between 70000 and 100000 frames at 15000 fps.

After recording, the XY trajectories of each particle were tracked using a custom-designed python program with subnanometer accuracy. Lastly, each obtained trajectory was analyzed employing another program that provided an estimated stiffness value from both X and Y directions. See FIG.1.

With regards to trap calibration, both the equipartition theorem and the power spectrum analysis were implemented. As far as we known, this was the first implementation of power spectrum analysis with a camera at such high frame rates.

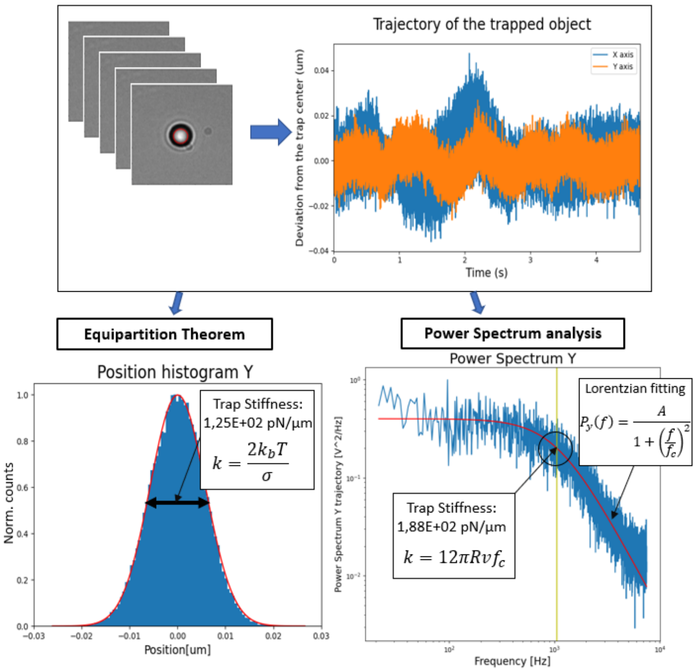

FIG. 1: Graphical description of all the steps performed to calibrate the trap stiffness. The trajectories corresponded to a high-speed video recording of 70000 frames at 15kHz.

B. Stability analysis of time-sharing optical tweezers

As previously described, the time-sharing method generates multiple optical tweezers by diverting a laser beam between different trap positions. This causes that for a given position the laser periodically turns on and off and as a consequence, the object exhibits free Brownian motion during the off time. Intuitively, the trapping stability would depend on the time-sharing switching frequency, since for slower switching frequencies the particle has more time to drift away.

To study the trapping stability of time-sharing optical tweezers, the dynamics of a single particle were analyzed for multiple different switching frequencies. The frequencies ranged from 50Hz to 50kHz, from the particle feeling the laser switching to the trapped particle feeling a constant intensity trap.

Lastly, a plot of the trap stiffness evolution along with the switching frequency was done for the equipartition theorem and the power spectrum. See FIG.1.

C. Multiple trap generation using AODs : Time-Sharing VS Holographic approach

Time-sharing and holographic optical tweezers are equally effective in generating a few optical traps. However, as the number of traps increases, the time-sharing technique begins having issues in terms of stability.

In this experiment, N different traps were created using both techniques and the stiffness for each of the trapped objects was calibrated. The objective was to confirm that when the switching frequency is fast enough, the trapped objects feel the average laser intensity.

To conduct the experiment, a program was developed to generate RF signals which would then be synthesized, amplified, and sent to the AODs using an arbitrary waveform generator AWG (Spectrum M41 6631) and a set of custom RF amplifiers.

On one hand, to create the time-shared optical traps, the custom program generated different pure sinusoidal signals (each one with its own frequency depending on the position of the trap) at different repetition rates into the AWG.

On the other hand, to create holographic optical tweezers, the custom program generated one RF signal equivalent to the sum of each pure sinusoidal signal (each one corresponding to a trap) into the AWG. However, by using this superposition method, the diffraction efficiency decreased significantly. To solve this issue, an optimization algorithm for the superposed signal was implemented: the algorithm consisted in changing the phase of each frequency, allowing the creation of a signal with the same frequency content but with an efficiency 2 to 10 times higher, see FIG.2. The final signal had the following form:

$$s(t) = \sum_{i=1}^{5} \sin\left(2\pi f_{p_i} t + \varphi_i\right) \tag{2}$$

Where φ_i is the obtained phase after the optimization and f_{p_i} is the position-dependant acoustic frequency.

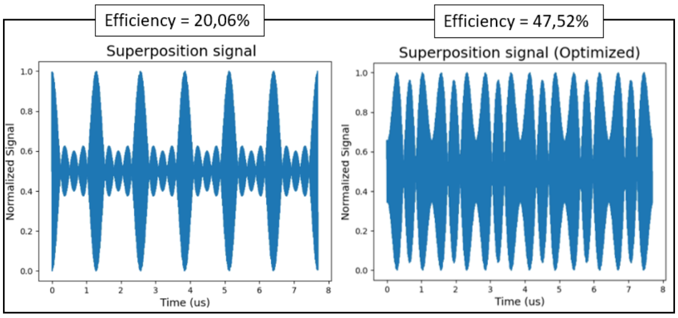

FIG. 2: Light efficiency improvement of the superposed signal with the optimization algorithm for the 5-trap case.

Once the process was completed, the acquired recordings were processed by the tracking software and the stiffness of the traps for both techniques was measured and compared. See FIG.3.A.

D. Acusto-holographic optical tweezers: custom two trap configuration

In this experiment, an alternative method to perform multiple optical traps was studied. This method is named Acousto-Holographic Optical Tweezers (AHOT) and consists of a combination of time-sharing and holographic optical tweezers. The method allows us to create permanent holographic traps whose configuration can be modified at extremely high-speed rates.

A two-trap configuration was implemented (a trap dumbbell, widely used in DNA stretching experiments), created from fast switching between a single trap and two permanent traps. This scheme was introduced as an extra option in the software used in section C.

The AHOTs were used to trap two micro-spheres so that one was trapped permanently and the other intermittently. To achieve that, the command introduced in the software was a periodic input that sent two signals sequentially. The first was a pure sinusoidal RF signal that generated a permanent trap for a given position, and the second was a superposed signal of two pure sinusoidal signals that generated holographic traps: one at the same position as the permanent trap from the first signal and the other at another given position. See FIG.3.B.

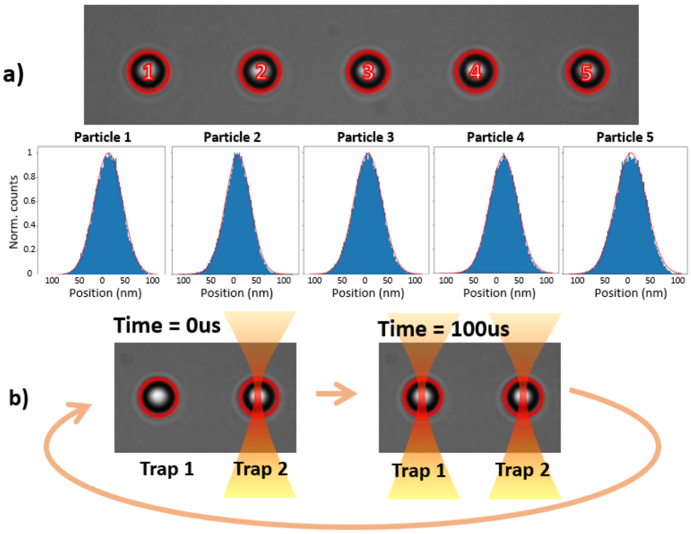

FIG. 3: a)Position histograms of the five trapped particles by HOTs from section C. b)Trap configurations of AHOTs described in section D.

III. RESULTS

The results obtained during the study of the stability of time-sharing optical tweezers are:

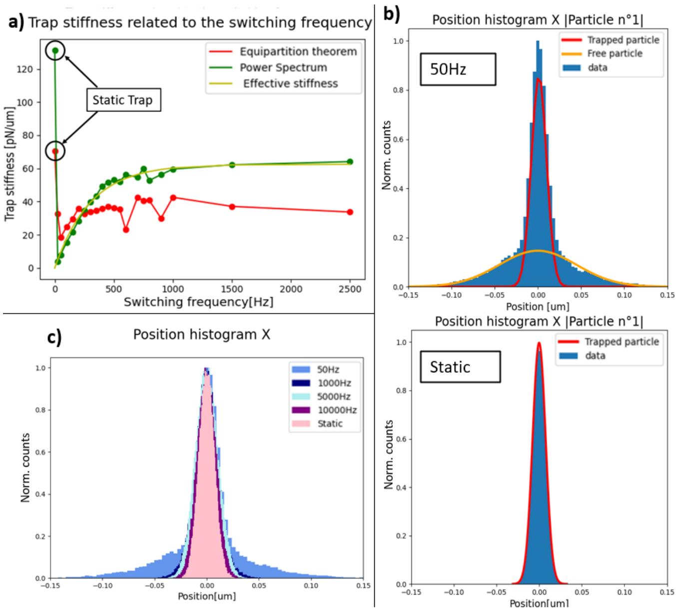

FIG. 4: a) Analysis of trap stiffness for different time-sharing frequencies. b) Comparison of the position histogram of a 50Hz time-sharing trap and a static trap. c) Evolution of the position histogram for different switching frequencies.

The first interesting result observed in FIG.4.A is that the stiffness values obtained by the equipartition theorem are significantly lower than those from the power spectrum analysis.

When applying the equipartition theorem, directly trap stiffness is measured from the thermal motion, while in the power spectrum it is measured in the frequency domain of the particle motion. In the real world, particle motion is affected not only by temperature fluctuations but also by uncontrolled external forces in the form of mechanical drifts. This effect underestimates the stiffness calibration for the equipartition theorem, whereas in the case of the power spectrum analysis, low-frequency drifts can be easily filtered out in the frequency domain. As seen in the values obtained, there is more than a 2x discrepancy between both calibrations. This is the reason why in this work, the power spectrum was implemented with a high-speed camera, because it is a more accurate method than the equipartition theorem.

In both cases, the trap stiffness as a function of the switching frequency follows the same tendency and matches the theoretical simulations with the Box Lucas model, in which this dependency is expressed as follows [9]:

$$k_{eff} = k_0(1 - exp(-f_{sw}/f_{ch}) \tag{3}$$

Where k_{eff} is the trap stiffness, k_0 is the transient-free stiffness (or in other words the stiffness corresponding to a permanent trap, f_{sw} is the switching frequency and f_{ch} is the the trap characteristic frequency.

The drifting phenomenon from using the time-sharing approach can be observed in the position histograms of the analyzed trajectories in FIG.4.B. For the permanent trapped case, the histogram is a pure Gaussian distribution as expected from the equipartition theorem, whereas in the case of time-sharing, the histogram starts to differ from a Gaussian distribution as soon as we reduce the switching frequency. For lower switching frequencies, the position histogram can be modeled as two Gaussian distributions corresponding to the periods where the trap is on and off. It is worth mentioning that this can only be seen thanks to the very high acquisition speed of our camera.

Looking at FIG.4.A again, this phenomenon can also be observed and allows us to distinguish between two different frequency regimes. For frequencies higher than f_{ch} the particle feels an average laser intensity which corresponds to 1/2 of the static trap stiffness. On the other end, for lower frequencies, we can see that the particle starts to feel the laser blinking, and thereby the particle responds with a lower effective stiffness. As expected, the limit between both regimes is located at f_{ch} which is the characteristic frequency of the trap.

The results from the study of the different multi-trapping techniques are as follows:

	Trap stiffness (pN/μm)				
	Trap 1	**Trap 2**	**Trap3**	**Trap 4**	**Trap 5**
Holographic	6.1	6.2	5.7	7.6	5.4
Time-Sharing	16.00	15.4	14.4	16.6	17.0

TABLE I: Trap stiffness values generated by time-sharing and holography techniques in section C.

The trap stiffness values from TABLE I. confirm that if f_{sw} is high enough all five particles average the laser intensity for the time-sharing approach. Of course, in the holographic case, the traps are static and the intensity is already distributed between the 5 different traps. However, trap stiffness from the holographic technique is lower than that from time-sharing, this is due to the modulation of the RF signal caused by the superposition of pure sinusoidal signals, which results in a lower optical efficiency. The slight variation between the 5 different stiffness in the holographic approach is attributed to the fact that the AODs do not transmit the laser with the same power in all directions.

At last, in TABLE II we show the different stiffness for each of the 2 traps generated by the 3 different techniques: time-sharing, holography, and a combination of both (AHOT).

Like in the previous experiment, we can see that for both holographic and time-sharing approaches the stiffness of both targets are more or less equal since the trapping laser is divided either temporarily or spatially.

Interestingly, in the proposed AHOTs configuration

	Trap stiffness (pN/μm)	
	Trap 1	**Trap 2**
Holographic	15.5	19.3
Time-Sharing	32.2	34.4
Acousto-Holographic	8.33	51.0
Acousto-Holographic (Adjusting power)	9.71	14.6

TABLE II: Trap stiffness values of the two traps generated depending on the multi-trapping technology being used.

the stiffness in trap 2 is higher than in the other. It should be noted that the stability of trap 2 is much higher than trap 1, this is due to particle 2 being always trapped while the laser at position one, is blinking. The trap in position 2 is fast switching between 2 different intensities, one at 100% (all the energy is concentrated in one spot) and the other at 25% (where 50% is lost at the hologram efficiency which then splits into two spots). On the other side, in trap 1, the laser power is switching between 0 and 25%. Theoretically, on average trap 2 is 5 times more intense than in trap 1. This is in agreement with the obtained stiffness values for both objects.

In the last proposed case of TABLE II, we reduced the power of the pure sinusoidal. Resulting in the laser power remaining constant all the time in trap 2, whereas in trap 1 the laser power was blinking.

IV. CONCLUSIONS

The trap stiffness, and thereby trap stability, in time-sharing optical tweezers strongly depends on the switching frequency of the trapping laser. This drastically compromises trapped object stability when increasing the number of trapped elements. For frequencies above the characteristic frequency (i.e. the particle's response time) the trap is stable. Even though the laser is jumping between N different positions, each particle responds exactly the same way as being trapped by a permanent laser and responding to external forces with an average stiffness equal to k_0/N, where k_0 corresponds to the stiffness of a continuous wave laser. However, for frequencies below that characteristic frequency, the trapped object starts to respond to the laser flickering, becoming more unstable as we reduce the switching frequency, or in other words, as we increase the number of targets.

If the switching frequency of the time-sharing is high enough, time-sharing optical tweezers average the laser intensity just as in the case of holographic optical tweezers. However, while using AODs to generate both techniques, the stiffness values from the holographic traps will be lower than the stiffness from the time-sharing traps.

Acousto-holographic optical tweezers are an alternative approach to trapping multiple objects that combines the generation of multiple permanent trap groups with the fast switching capabilities of AODs. Permanent traps improve the object stability, while fast switching to other target positions allows addressing single object information. With the same AOD-based optical setup, both static and time-shared optical traps can be created, and the 2-trap configuration proposed in this work is a clear example of AHOT capabilities. Note that in a pure holographic approach, no forces can be measured, whereas in a time-sharing scheme, objects become less stable, compromising the precision of force measurements. However, in this AHOT approach, object 2 is constantly trapped but the force at that position can still be directly measured.

V. ACKNOWLEDGMENTS

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