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Dynamic axial stabilization of counterpropagating beam-traps with feedback control

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Abstract: Optical trapping in a counter-propagating (CP) beam-geometry provides unique advantages in terms of working distance, aberration requirements and intensity hotspots. However, its axial performance is governed by the wave propagation of the opposing beams, which can limit the practical geometries. Here we propose a dynamic method for controlling axial forces to overcome this constraint. The technique uses computervision object tracking of the axial position, in conjunction with softwarebased feedback, for dynamically stabilizing the axial forces. We present proof-of-concept experiments showing real-time rapid repositioning coupled with a strongly enhanced axial trapping for a plurality of particles of varying sizes. We also demonstrate the technique's adaptability for real-time reconfigurable feedback-trapping of a dynamically growing structure that mimics a continuously dividing cell colony. Advanced implementation of this feedback-driven approach can help make CP-trapping resistant to a host of perturbations such as laser fluctuations, mechanical vibrations and other distortions emphasizing its experimental versatility.

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1. Introduction

Optical trapping [1] systems find themselves in a myriad of experimental settings that range from fundamental research such as atom trapping to applied sciences such as biophotonics, biophysics and nano-sciences to mention a few. Many trapping schemes are based on optical tweezers that require high numerical aperture (NA) oil-immersion objectives [2]. This high-NA geometry sets stringent aberration requirements that limit applicable sample chambers. The available working distance (a few hundred microns) sets a physical limit to the realizable axial trapping range even when aberrations are minimized (e.g., by adaptive correction [3], or by replacing the oil-immersion with water-immersion objectives [4,5]). This is a pity, since lower aberration sensitivity and a longer working distance can provide the experimentalist with a wider latitude for optical trapping and manipulation, such as when selecting or designing sample chambers and adding auxiliary instrumentation. Moreover, a high-NA geometry creates a tight focus within the trapped object that could initiate unwanted side effects for living organisms. With the advent of micro/nanostructure fabrication facilities, such as two-photon photo-polymerization, a less constrained optical trapping system is desirable for maneuvering micro/nanostructures [6-8] for applications in fields of microrobotics, micro-assembly, nano-surgery etc (e.g. to probe living cells from a plurality of directions, or conduct various micro-spectroscopy approaches [9]).

To avoid the aforementioned limitations, we turned our efforts towards developing the counter-propagating (CP) beam-trapping geometry for simultaneously handling multiple particles dynamically and independently. The use of low-NA objectives in the CP geometry affords a large working distance, sets less stringent constraints on the sample chamber, and traps particles without sharp focusing. Some CP geometries are illustrated in Fig. 1.



Fig. 1. a) Conventional stable CP-trapping in the far-field of diverging beams. b) Overlapping foci. c) Converging beams with foci oppositely positioned compared to a). d) CP-trapping with tube-like beams.

Two diverging beams create a stable CP trap in Fig. 1(a). Pioneered by Ashkin [1], it has been adapted in fiber-based [10] and GPC-based [11,12] systems. Axial manipulation is

achieved by varying the intensity ratio between the beams. The low axial intensity gradient of the far-field beams leads to low axial stiffness and sluggish axial motion. Moreover, light that spills outside the particle wastes energy and can even interfere with neighboring traps. Spilled light is minimized when the foci overlap as in Fig. 1(b). This improves transverse stiffness and creates a very strong trap, even for highly scattering objects, using high-NA [13], but can become unstable when minimizing intensity hotspots using lower NA. It also needs axial focal shifting for axial manipulation. The converging beams in Fig. 1(c) also create unstable traps [14], although it can be stabilized by alternating it with Fig. 1(a) [15]. The tube-like beams in Fig. 1(d) maintains optimal transverse gradients over very long operating distances but is generally unstable since the axial forces cancel, though subwavelength particles may be trapped and transported over hundreds of microns using standing wave gradients [16].

Given the strengths of a counterpropagating geometry, how can one work around some of its weaknesses? For example, the stability and stiffness of the CP geometry is sensitive to the foci separation [11,14] since it needs a proper axial variation of the opposing axial forces that, in turn, depends largely on the wave propagation. One way, therefore, is to synthesize light fields that have desirable propagation properties [17]. In the present work, we adopt a dynamic approach to control the axial forces and improve the stability of CP traps. We apply active stabilization [18,19] using a vision-feedback system that monitors the axial particle position with a side-view microscope and then regulates the intensity of the opposing beams accordingly. We also use computer-vision and software-based feedback to track and trap an array of particles. When the previously unstable CP-geometries can be used in a highly controlled way, one can have a merit-driven choice of CP-trap geometry, for instance to minimize hotspots on living cells using Figs. 1(a), (c) or optimize transverse forces using Figs. 1(b), (d). This also avoids having to adjust the focal separation between the opposing beams for different particle sizes, as required in static CP-beams [11]. Furthermore, our software-based approach can be combined with other trapping schemes that apply machine-vision [20].

We describe our experimental setup and the computer-based feedback implementation in section 2. We present our experimental results in section 3 where we illustrate the principle of dynamic axial stabilization at work for optical trapping and manipulation of single, multiple, chained and multiple-sized particles over an axial range of 250 microns. We summarize our findings and present an outlook in the last section.

2. Experimental setup and side-view vision-feedback implementation

The experiments were performed using our in-house developed BioPhotonics Workstation (BWS), which is illustrated in Fig. 2. The BWS configuration and optical modules have been previously described in detail [9], so we outline only the pertinent features here. Two independently addressable regions of a spatial beam modulating module are optically mapped and relayed as a plurality of reconfigurable counter-propagating beams in the sample. The scaling between the spatially light modulating pixels and the sample plane are specified by choosing appropriate focal lengths of the relaying lenses. The user can independently control the number, size, shape, intensity and spatial position of each CP-beamlet through a LabVIEW interface. Each CP-beamlet can independently trap and manipulate a plurality of microscopic objects. Although we verified that feedback works for the different geometries depicted in Figs. 1(a)-(c), the results presented in the next section are based on the diverging CP-beam geometry in Fig. 1(a).

The wide working space between the barrels of two objective lenses (Olympus LMPLN 50 \times IR, WD = 6.0 mm, NA = 0.55) easily accommodates a 4.2 mm thick sample chamber (Hellma cytometry cell, 250µm \times 250 µm inner cross-section, 1.6µL volume). Fluid borne polystyrene beads (5 µm and 10 µm diameters) are loaded into the Hellma cells. The cells have optically flat surfaces that are suitable for trapping and imaging. The available working space enables other imaging modalities to be easily attached orthogonally, such as fluorescent microscopy, thus enhancing the versatility of the instrument. In this work, a side-view microscope monitors the axial positions of the trapped particles (see Fig. 2). We use this unique observation mode, usually unavailable in optical tweezing, to provide real-time

position feedback for active stabilization. Images from the side-view video microscope are streamed to a computer for particle tracking and analysis. The feedback software and multiparticle tracking algorithms are developed in LabVIEW using its built-in image and vision processing features. Since our approach is purely software-based it can be easily adapted for a variety of trapping configurations. A key feature is that multiple particles, even of varying sizes, can be rapidly trapped and repositioned simultaneously.



Fig. 2. Schematic of active stabilization in the BioPhotonics Workstation (BWS). An array of actively regulated traps are relayed through well-separated objectives (1 and 2) that provides ample space for side-view microscopy (objective lens A, zoom lens and CCD camera). Computer vision provides real-time position feedback for regulating the traps.

The following procedure describes our real-time feedback approach for stabilizing a counter-propagating trap located at a user-defined and reconfigurable transverse position:

- 1. The user inputs the desired axial position, Z_d , for a particle (screen pixel coordinate).
- 2. The side-view computer-vision finds the particle position, Z_m , and its error, $\varepsilon = Z_m Z_d$.

3. The controller compares the error, ε , with two thresholds, ε_{max} and ε_{min} , and sets the respective intensity control signals, I_{\uparrow} and I_{\downarrow} , for the upward and downward beamlets:

- $|\varepsilon| > \varepsilon_{\text{max}}$: Set the beamlet pushing toward Z_d to maximum, I_{max} , and turn off the opposing beam for laser-catapulting the particle towards the desired position.
- $\varepsilon_{\min} < |\varepsilon| < \varepsilon_{\max}$: Set the correct beam at I_{\max} and the opposite beam to $I_{\max} \Delta I$.

• $|\varepsilon| < \varepsilon_{\min}$: Set both beams to I_{\max}

Thus, we have a simple tri-state controller where the 255-level intensity control signal can be 0, 240, or 255, with thresholds $\varepsilon_{max} = 5$ pixels (1.35 µm) and $\varepsilon_{min} = 3$ pixels (810 nm). These steps are looped (processing 30 frames per second) for active stabilization and error suppression. For multiple particles, a tracking system ensures correct addressing of axial positions while steps 1 to 3 are simultaneously executed for each particle.

3. Experimental demonstrations and results

As a first demonstration we trapped and axially manipulated a 10 μ m diameter particle, initially lying on the bottom, over the entire channel height. Side-view video microscopy (Media 1; see snapshots in Fig. 3) reveals the particle dynamics arising from the feedback algorithm. The dynamic modulation of the beamlet intensities in the CP-trap is able to position the particle even at previously unstable locations. When the particle moves away from its desired position, the error is detected by the side-view tracking software and the feedback loop restores the particle to its desired position. Even when the focal positions are shifted by axial chamber displacements, active stabilization returns the particle to the desired position. The results also show that the jitter varies depending on the selected axial position. Results show that the particle appears locked for some desired positions, with jitter of a few pixels, and exhibits larger oscillations for other positions. This is an artifact of the simple tristate control that does not account for the axial variation of the beamlet intensity and can be improved by using non-diffracting beams or a more sophisticated control system.

As mentioned in the introduction, it is cumbersome to adjust the beamlets foci separation for trapping different particle sizes with static CP-traps. To demonstrate that our feedback approach is insensitive to the foci separations, we used it to trap and manipulate 5 and 10 μ m diameter particles simultaneously. To optimize the photon flux through each particle, the transverse beamlet sizes and shapes are optimized in the spatial light modulating module to fit the morphology of each particle. The advantage of a feedback-based approach for multi-sized particle trapping is evident from the experimental results in Media 2 in Fig. 4.



Fig. 3. Media 1. Snapshots from side-view microscopy of optical manipulation and trapping of a 10 μ m diameter particle using an actively stabilized counterpropagating-beam trap. The blue rectangle overlay is centered on the desired position. A red square circumscribes the auto-detected particle; a red dot marks its center-of-mass for position feedback.



Fig. 4. Media 2 Snapshots from side-view video microscopy of simultaneous optical manipulation and trapping of differently sized particles (5µm and 10µm diameter). Particles are rapidly trapped and stabilized via side-view feedback with static foci separations

Multiple particles can form a chain in a counter-propagating beam trap as the particles modify the surrounding optical field to trap nearby particles [21]. The neighboring particles would sense a force which is dependent not only on the impinging trapping laser light but also on the scattered light from its nearest neighbors in the chain. We observed that, even when multiple particles line up in a beam, the whole string of particles can still be rapidly repositioned using our side-view feedback technique. Intriguing dynamics is observed, which calls for a more elaborate theoretical modeling of such a dynamic light-matter interacting system. This shows that the feedback control technique can adapt to any kind of overall object shape, for example, when trapping colonies of dividing cells [22], which tend to arrange themselves into a variety of unpredictable configurations. Experimental results show the adaptability of our side-view feedback algorithm to such constantly changing trapping environments (see Fig. 5).

Optical manipulation of a plurality of particles is valuable, among others, for handling micro/nano-structures with multiple optical handles [6-8]. Another utility of feedback-based trapping is that multiple particles can be positioned independently in a volume. Side-view video microscopy demonstrates the independent repositioning and formation of axial particle configurations (see Fig. 6) using a simple on-off controller (i.e., control intensity signal is either 0 or 255, depending on whether the particle is above or below its desired position). The technique works even with the simpler controller, albeit with increased particle oscillations. Aside from forming various particle configurations, we also tested the robustness of stabilized

multiparticle trapping to focal shifts by again axially shifting the sample chamber. The results in Fig. 6 also display the close proximity one can have with the trapped particles over a large axial range. With traditional static divergent CP-trapping beams the unavoidable light spilling can create destabilizing cross-talk between adjacent traps.



Fig. 5. Media 3 Side-view microscopy showing optical trapping and manipulation of a chain of 5 µm diameter particles formed by optical binding: (a) The chain is catapulted toward desired position (blue overlay); (b) The chain of particles is held in place by dynamic traps.



Fig. 6. Media 4 Side-view microscopy showing simultaneous optical trapping and manipulation of multiple 10 µm diameter particles into various configurations using actively stabilized counter-propagating traps.

4. Conclusions

A simple, yet valuable enhancement to the conventional counter-propagating beam trapping approach is proposed and demonstrated with proof-of-principle experiments. The concept is based on computer-feedback with the aid of side-view vision processing for particle tracking enabling us to enhance the axial trapping range, repositioning speed and stability of a plurality of differently sized and shaped traps. This spatio-temporal modulating approach can be easily adapted to many other configurations. We have demonstrated trapping of multiple particles of varying sizes over a 250 µm micro-fluidic channel without concerning about the focusing geometry of the CP-trapping beams. This opens the door for true volume-oriented trapping and manipulation where real-time rapid repositioning and stabilizing of a plurality of particles are demonstrated. Our experiments also exhibit the adaptability of this technique to stably trap complex objects of dynamically varying composition such as growing chains of particles. We also showed that the method is robust against substantial external perturbations and can effectively re-capture objects that are temporarily lost from a given trap. Looking ahead, we anticipate its potential applications in supporting various trapping contexts, such as a) trapping with novel CP geometries optimized according to some merit function b) coping with aberrations, beam-misalignments, and perturbations such as mechanical vibrations, laser power fluctuations and drift. The precision limit of the technique is, hence, worth exploring.

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