

Development of Rapid Feedback and Optimized Detection Algorithms to Enhance the Spatiotemporal Resolution of the ABEL Trap

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ABSTRACT

This work presents a customized real-time particle tracking algorithm integrated with a Raspberry Pi-based feedback voltage controller to enhance the spatiotemporal resolution of the Anti-Brownian Electrokinetic (ABEL) trap. Unlike conventional ABEL trap implementations that rely on fluorescence modulation and specialized optical excitation geometries for particle localization, the proposed framework employs direct fluorescence imaging and real-time image processing to determine particle position, resulting in a simpler and more accessible feedback architecture. Using open-access video datasets for validation, our system achieved rapid feedback with a maximum latency of just 100 μ s, more than 10 times faster than previous reports while maintaining voltage accuracy within 4% of the simulated values. This rapid feedback has the potential to offer more stable and longer nanoparticle confinement, making it highly suitable for delicate single-molecule studies. Our findings highlight how the simplified sensing architecture, precise voltage control, and optimized detection algorithms can substantially boost ABEL trap performance, paving the way for real-time tracking of faster and smaller biomolecules.

Keywords: optofluidic, ABEL trap, electrophoresis feedback single molecule trapping.

1. INTRODUCTION

Single-molecule techniques have revolutionized our understanding of dynamic processes by revealing a breadth of information, heterogeneities, and transient states that are inaccessible in typical bulk ensemble measurements [1]. Trapping individual molecules represents a foundational strategy in this regard that allows for precise control over the analytes of interest and prolonged observation timescales critical for quantitative analysis in a variety of disciplines, including biophysics, engineering, and molecular diagnostics. Optical trapping, pioneered by Arthur Ashkin in the 1970s, remains a method of choice for trapping and manipulation of single molecules [2]. Using photon momentum transfer, this approach non-invasively confines particles from micro to nanoscale, with the stability needed for a diverse range of applications, including force spectroscopy and live-cell biophysics [3], [4]. However, traditional optical traps often suffer from poor scalability for smaller particles and require high optical power, which can damage the particles and limit their effectiveness for nanoscale applications [5]. With recent advancements, miniaturized lab-on-chip technologies are becoming popular that enable integrated trapping platforms which offer enhanced functionality and

flexibility by combining multiple techniques on a single platform [3], [4], [6]. Researchers have demonstrated various on-chip particle manipulation methods, such as optical tweezers [7], magnetic tweezers [3], [4], Photonic crystal fibers [5], [6], [8] and DNA curtains [9]), which provide precise control but are limited by issues like heat, low throughput, and specificity. Ring resonators [10], [11], Acoustic trapping [12] and dielectrophoretic methods [13] also face challenges related to integration and system complexity.

In recent years, the Anti-Brownian Electrokinetic (ABEL) trap has gained significant attention and positioned itself as a cutting-edge technique that enables precise single-molecule confinement by combining fluorescence-based tracking with electrokinetic feedback [14], [15]. Unlike optical tweezers that require intense illumination (often watt-level lasers) to generate sufficient gradient forces, the ABEL trap uses electrokinetic forces to counteract Brownian motion, requiring only microwatt-scale optical power for particle tracking [14], [15]. This dramatically reduces photodamage risks which is a critical advantage when studying delicate specimens like fluorescent proteins, live cells, or photosensitive nanomaterials. Since its first demonstration by Cohen

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and Moerner, the ABEL trap has enabled trapping of diverse biomolecules including viruses, DNA, and even small molecules (~ 1 kDa) in aqueous solution revealing its unique capability to confine nanoscale species that challenge conventional techniques. [3], [7]. While ABEL traps have demonstrated remarkable capabilities for single-molecule studies, their performance could be significantly enhanced by improving two critical factors. Firstly, improving particle tracking precision to precisely determine the particle position and track rapid Brownian motion, and secondly, faster voltage application to maintain stable trapping. These improvements are crucial because the effectiveness of an ABEL trap relies on rapid and accurate compensation of Brownian motion; any delay in particle localization or application of corrective voltages increases the likelihood of particle escape, thereby reducing trapping efficiency. Consequently, there is a need for feedback methodologies that not only minimize latency but also simplify the particle localization and control architecture while maintaining accurate real-time electrokinetic feedback. Such advancements would enable more stable confinement of delicate biological samples and expand the applicability of ABEL traps for investigating dynamic molecular processes that are currently challenging to study.

In this work, we present an improved ABEL trap methodology by integrating a custom Python-based particle tracking algorithm with a Raspberry Pi-based feedback voltage application. Building upon previous ABEL trap implementations, the proposed method employs direct fluorescence imaging and real-time image processing for particle localization instead of fluorescence modulation-based position estimation. This simplifies the sensing and feedback architecture while preserving the underlying electrokinetic trapping principle and enabling rapid real-time feedback. Our methodology was validated using published ABEL trap video datasets from Cohen and Moerner [16], Kuhn et al. [14], and Rahman et al. [17], demonstrating tracking precision and response times sufficient for stable nanoparticle confinement. Following video frame extraction, our Python-based image processing algorithm performs real-time particle tracking to determine the particle position. Upon successful tracking of the particles, corresponding bidirectional feedback voltages are generated by the hardware which in theory should drag the particle to the desired trapping position with a stable 2D confinement, an approach following the electrokinetic feedback principle reported by Rahman et al. [17].

2. METHODOLOGY

The ABEL trap is typically implemented by combining real-time optical detection with feedback control to counteract the Brownian motion of particles in solution, as illustrated in Fig. 1. A fluorescent molecule or nanoparticle is continuously imaged to determine its position, and a feedback system applies electrokinetic forces through microfluidic electrodes to push the particle back toward a defined trap center. This requires precise synchronization between optical measurements, position estimation, and voltage application. In the foundational work by Cohen and Moerner [16], the ABEL trap was realized using wide-field fluorescence microscopy coupled with real-time position estimation and electric field control executed on a custom-built software. Building on this concept, Kuhn et al. [14] developed a one-dimensional ABEL trap integrated onto a planar optofluidic chip that combined optical excitation, fluorescence detection, and electrokinetic feedback within a single microfabricated platform, as shown in Fig. 1(a). Their system employed solid-core waveguides embedded in the chip to deliver low-power laser excitation directly to the microfluidic channel, significantly reducing the optical power required for single-particle detection. Fluorescence emitted by the trapped particles was collected through the same chip-based architecture using a highly sensitive photodetector placed off-chip. Position estimation was based on the modulation of fluorescence intensity as the particle moved relative to the optical excitation pattern, which enabled real-time tracking.

Electrodes integrated along the microchannel applied feedback voltages to generate electroosmotic flow, effectively suppressing Brownian motion and keeping the particle confined near the trap center. This approach demonstrated the feasibility of miniaturized, power-efficient ABEL trapping, offering a scalable platform for portable single-particle analysis. Rahman et al. [17] further extended this concept by implementing two-dimensional electrokinetic feedback using intersecting optical waveguides, as illustrated in Fig. 1(b). Similar to Kuhn et al. [14], particle localization relied on fluorescence modulation, with the resulting position information used to generate feedback voltages that restored the particle to the trap center. The fully integrated optofluidic architecture provided a compact solution for single-particle trapping; however, the reported feedback latency remained on the order of a few milliseconds, limiting the trapping of smaller and faster-diffusing particles. Reducing this latency while simplifying the sensing and feedback architecture remains an important direction for improving ABEL trap performance.

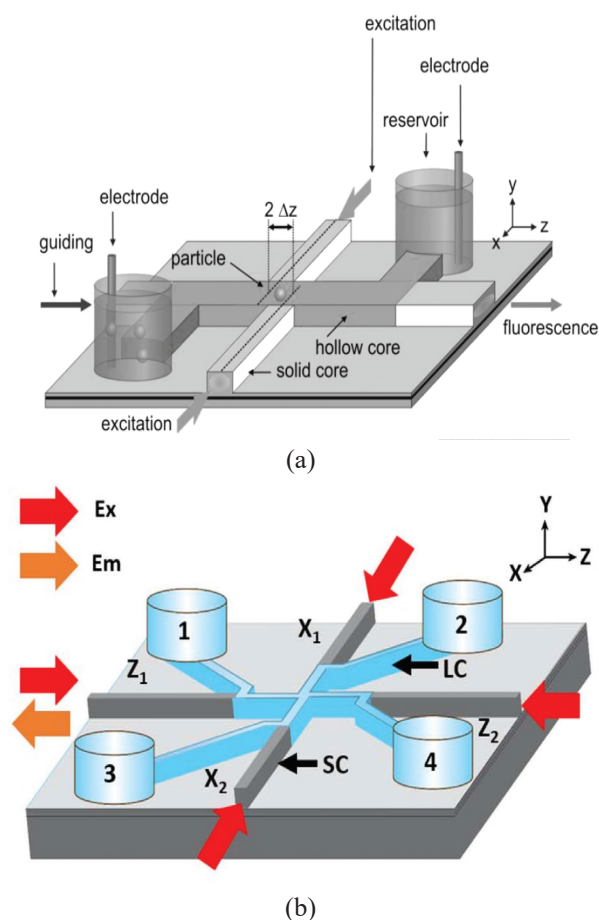


Fig. 1: Schematic representation of two ABEL traps designs: (a) simplified configuration introduced by Kuhn et al. [14] and (b) conventional configuration as presented by Rahman et al. [17].

Since rapid feedback is critical for efficient trapping particularly for smaller and more diffusive particles, it is essential to implement both fast voltage control and accurate particle tracking. To address this need, we have envisioned a methodology suitable for integration into typical on-chip systems, where fluorescent images of particles can be captured using a high-speed camera, such as a CCD (see Supplementary Section S1 for the proposed system architecture). Similar to conventional ABEL trap methods, our approach relies on image-based tracking coupled with a custom-developed algorithm to determine the particle's position in real time. Similar to Rahman et al. [17], our method assumes an anti-resonant reflecting optical waveguide (ARROW) platform featuring intersecting liquid-core waveguides, which facilitate analyte introduction through a standard glued reservoir. Unlike previous methods demonstrated by Kuhn et al. [14] and Rahman et al. [16], which rely on offset excitation waveguide geometries and periodic ON–OFF modulation

of excitation beams, our technique does not require such configurations. This significantly simplifies the optical setup and removes the need for complex fluorescence-based modulation schemes. Furthermore, a straightforward top-down excitation with low optical power can also be used to excite fluorescence in labeled particles, enabling effective tracking through standard fluorescence imaging and appropriate filtering. As a result, the proposed method has the potential to offer a more accessible and adaptable platform for high-resolution ABEL trapping. Since our proposed method relies on fluorescence imaging of particles, an approach consistent with the techniques used in the previously mentioned studies, we validated our tracking algorithm using fluorescence videos published in those works. These videos, which capture the motion of fluorescently labeled particles, were processed through a custom-developed Python algorithm designed to accurately determine particle positions in each frame. The tracking data were then analyzed on a Raspberry Pi, which computed two-dimensional corrective voltages in real time. These voltages represent the electrokinetic feedback required to drive the particle toward the designated trap center, thereby demonstrating the viability of our approach for precise particle manipulation. The voltages can then be applied to the analyte via standard Ag–AgCl electrodes. The subsequent sections provide a comprehensive description of the system components, methodologies, and data collection procedures.

A real-time image processing algorithm was developed using Python to enable precise tracking, analysis of particle dynamics, and electric field-based manipulation for particle trapping. The fundamental concept underlying the algorithm is outlined in the flowchart depicted in Fig. 2.

The process begins with high-speed video frame acquisition of the trapping environment. Each captured frame transforms the Hue, Lightness, Saturation (HLS) color space, followed by thresholding operations for isolating the target particle. Following isolation, the algorithm detects the contour of the identified particle and computes the corresponding centroid. A positional error is then calculated by comparing the computed centroid with a predefined arbitrary target location. The system continuously iterates this process, acquiring new frames, updating the position, and applying corrections and thereby the particle is confined.

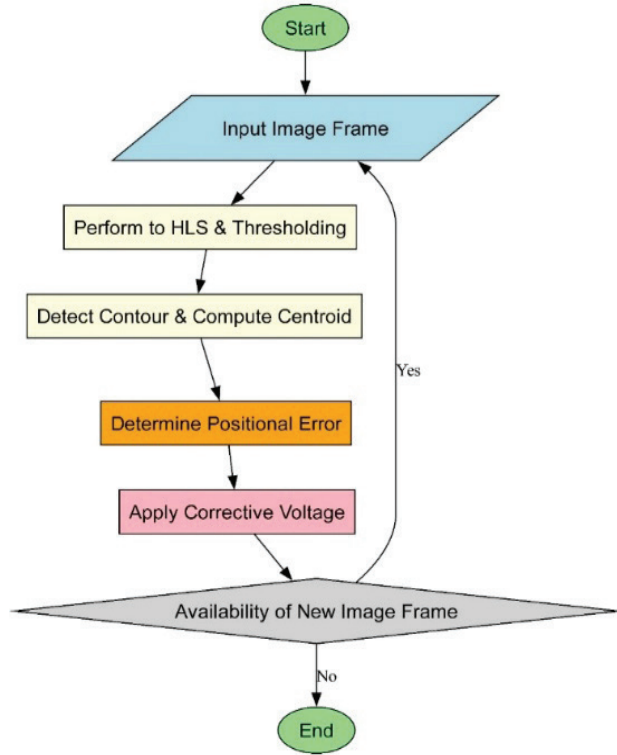


Fig. 2: Flowchart of the Real-Time ABEL Trap Biomolecule Tracking, Image Processing, and Voltage Adjustment Process

A predefined Region of Interest (ROI) is employed to localize the trapping region, thereby minimizing computational demand and enhancing algorithmic efficiency. Prior to system operation, the ROI is determined through an initial calibration stage to fully encompass the trapping region (see Supplementary Section S2 for further details). The ROI is mathematically represented as [18]:

$$ROI = \begin{cases} 1, & \text{if } x_{lower} \leq x \leq x_{upper} \text{ and } y_{lower} \leq y \leq y_{upper} \\ 0, & \text{otherwise} \end{cases} \quad (1)$$

Where denote the predefined spatial boundaries that enclose the region under analysis.

Enhanced segmentation of the target particle against the background is achieved by converting each frame to the HSV (Hue, Saturation, Value) color space. This representation enables more distinct separation based on intensity and saturation gradients. The RGB-to-HSV conversion follows these expressions [19]:

Hue (H):

$$H = \begin{cases} 0^\circ + 60^\circ \times \frac{G - B}{\max - \min}, & \text{if } \max = R \\ 120^\circ + 60^\circ \times \frac{B - R}{\max - \min}, & \text{if } \max = G \\ 240^\circ + 60^\circ \times \frac{R - G}{\max - \min}, & \text{if } \max = B \end{cases} \quad (2)$$

Saturation (S):

$$S = \frac{\max - \min}{\max} \quad (3)$$

Value (V):

$$V = \max \quad (4)$$

In these expressions, R, G, and B represent the red, green, and blue intensities, respectively, while H corresponds to hue in degrees, S denotes saturation, and V refers to the brightness component.

Using thresholding, a binary mask isolates particles with specific color profiles. The threshold values are selected during the initial calibration stage based on the fluorescence intensity and background characteristics of the acquired images to achieve reliable particle segmentation. Since these values depend on the imaging conditions, they are determined during the initial calibration stage prior to system operation (see Supplementary Section S2 for further details). The largest contour is then selected as the most probable particle candidate:

$$C = \arg \max (\text{Area}(C_i)), \quad \text{for } C_i \in S \quad (5)$$

Where, S represents the set of all detected contours, C is the contour with the largest area.

The centroid of the contour is computed to determine the precise location of the particle within the LC medium. The centroid coordinates (cX , cY) are calculated using the image moments:

$$cX = \frac{M_{10}}{M_{00}}, \quad cY = \frac{M_{01}}{M_{00}} \quad (6)$$

Where, are the spatial moments of the contour

The computed centroid coordinates, which are in pixel values due to video processing, are mapped to real-world values using a predefined scaling factor, allowing accurate positional tracking. The positional error is determined by calculating the difference between the detected particle position and a user-defined reference coordinate within the trapping region. The error is computed in terms of pixel distance from the trapping region coordinate:

$$E_x = X_{\text{ref}} - cX, \quad E_y = Y_{\text{ref}} - cY \quad (7)$$

Where, (X_{ref} , Y_{ref}) is the predefined target position for trapping.

A Raspberry Pi-based system performs real-time voltage control using the computed positional errors. Fig. 3(a) shows a sample video frame with a fluorescent biomolecule and the defined trapping point ($x = 14.25 \mu\text{m}$,

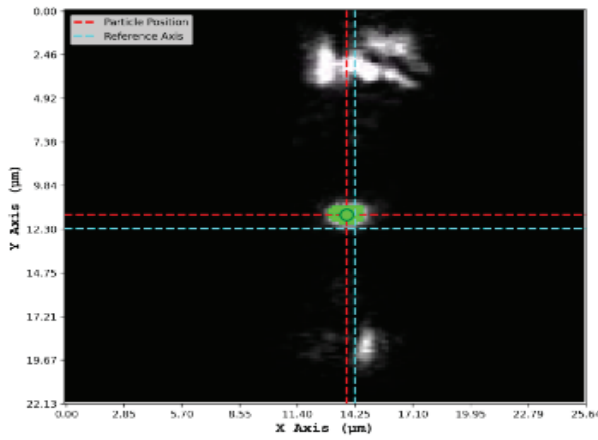
$y = 12.30 \mu\text{m}$). A maximum voltage of 3.3 V, of either polarity, is applied independently along the x- and y-axes. The voltages along these axes, denoted as V_x and V_y , are proportional to the positional error and are dynamically updated to steer the biomolecule toward the trapping point. This process is illustrated in Fig. 3(b).

Voltage adjustments follow:

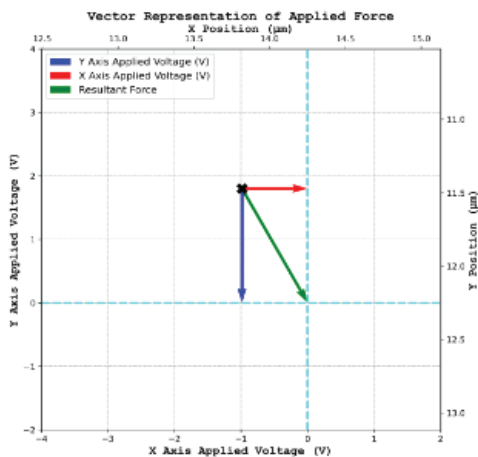
$$V_x = K_x \cdot E_x, \quad V_y = K_y \cdot E_y \quad (8)$$

Where, K_x and K_y are proportional gain constants selected during the initial calibration stage (see Supplementary Section S2 for further details).

The system continuously monitors subsequent frames, recalculating the positional error and adjusting the electric field until the particle reaches a static state.



(a)



(b)

Fig. 3: (a) Representative video frame extracted from Movie-3 in the supplementary material Khun et al.[14], illustrating the position of a fluorescent green biomolecule and the designated trapping point at $(x = 14.25 \mu\text{m}, y = 12.30 \mu\text{m})$. (b) Real-time voltage adjustment scheme for x and y-axis.

As shown in Fig. 4, the system utilizes a Raspberry Pi 5 receiving input image frames and two MCP4921 12-bit DACs, each paired with an amplifier for analog voltage output. The DACs, featuring a $4.5 \mu\text{s}$ settling time and 20 MHz SPI interface, convert digital control signals into analog voltages that are applied to the trapping electrodes. An oscilloscope is included in the setup for real-time monitoring of the output signals.

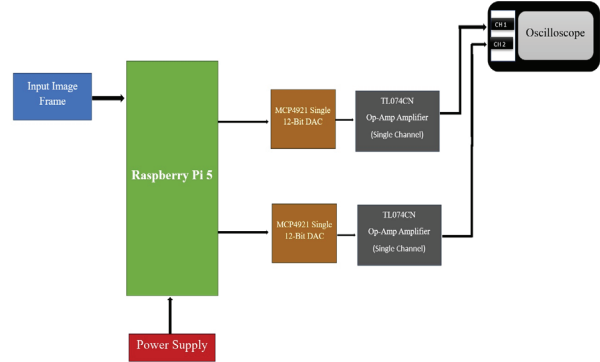


Fig. 4: A block diagram showing a Raspberry Pi 5-based system receiving input image frames and connected to two MCP4921 12-bit DACs and an oscilloscope for signal monitoring and analysis.

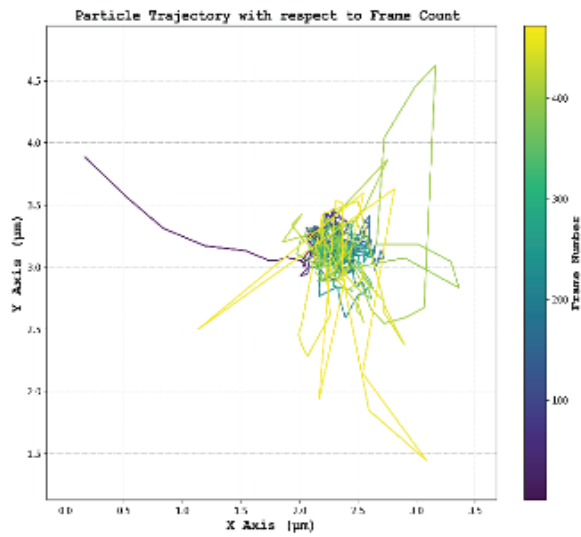
The trajectory of the trapped biomolecule is logged throughout the experiment. Both position data and voltage corrections are recorded and plotted to visualize movement patterns and the stabilization process. This real-time feedback system ensures accurate and dynamic trapping of particles within the ABEL trap, supported by high-speed imaging, efficient contour detection, and responsive field control.

3. RESULT AND DISCUSSION

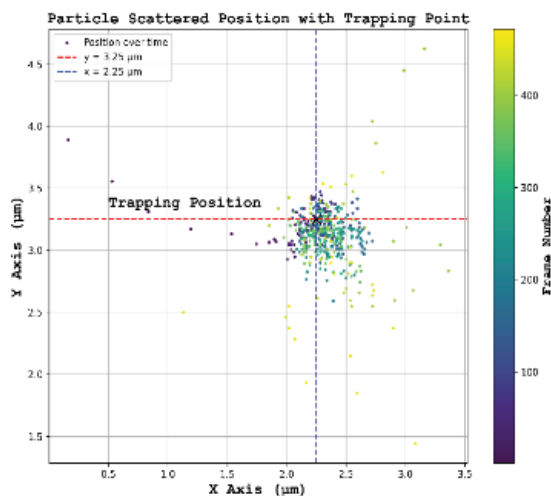
We validated the proposed image-processing-based ABEL trapping algorithm using publicly available videos from Cohen and Moerner [16] and Kuhn et al. [14], as outlined in the Methodology section, which simulate typical trapping conditions in ARROW optofluidic platforms.

In the first case, we utilized Movie-3 from the supplementary material of Cohen and Moerner [16], which demonstrates the tracking of individual large protein molecules such as B-phycoerythrin $\sim 800 \text{ nm}$ in diameter over 472 frames spanning 8 seconds. The trajectory in the x–y plane, as illustrated in Fig. 5(a), shows progressive localization toward a central region near $(x = 2.25 \mu\text{m}, y = 3.25 \mu\text{m})$, with a color gradient from purple to yellow indicating time progression across frames. A static

scatter plot shown in Fig. 5(b) further confirms stable confinement, with most positions constrained within approximately $0.3\ \mu\text{m}$ of the target location.



(a)

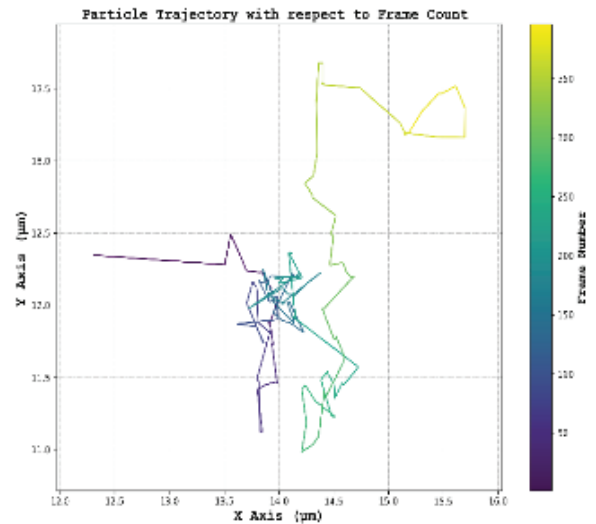


(b)

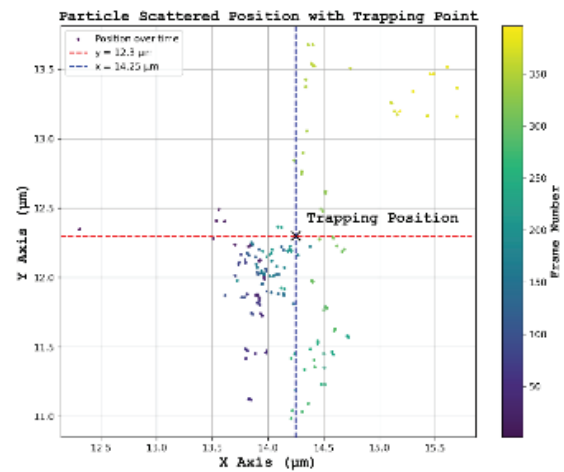
Fig. 5: Trajectory analysis of a large B-phycoerythrin protein molecule using the proposed image-processing algorithm. (a) Time-resolved trajectory extracted from the experimental video by Cohen and Moerner [16]. (b) Corresponding scatter plot showing spatial confinement near the trapping region.

Similarly, the trajectory extracted from Movie-3 in the supplementary material of Kuhn et al. [14] demonstrated consistent particle tracing within the designated trapping region. In this case, the trapped particle was a stained *Escherichia coli* (*E. coli*) bacterium. The trajectory, illustrated in Fig. 6(a) using a frame-color gradient, exhibits continuous tracking over 395 video frames. The x - y scatter plot shown in Fig. 6(b) confirms successful position detection near the trapping coordinate at ($x =$

$14.25\ \mu\text{m}$, $y = 12.30\ \mu\text{m}$). The particle remained confined within the $\sim 2\ \mu\text{m}$ excitation region throughout the duration of the sequence.



(a)



(b)

Fig. 6: Trajectory analysis of a stained *E. coli* bacterium using the proposed image-processing algorithm. (a) Time-resolved trajectory extracted from the experimental video by Kuhn et al. [14]. (b) Corresponding scatter plot showing spatial confinement near the trapping region.

Despite the variability in particle dynamics across the two video sources, the image-processing algorithm demonstrated robust and consistent real-time tracking performance. The algorithm achieved accurate frame-by-frame detection and localization of *E. coli* bacteria and B-phycoerythrin protein molecules throughout the recorded sequences, thereby validating the applicability and effectiveness of the proposed method across a broad range of biomolecular targets.

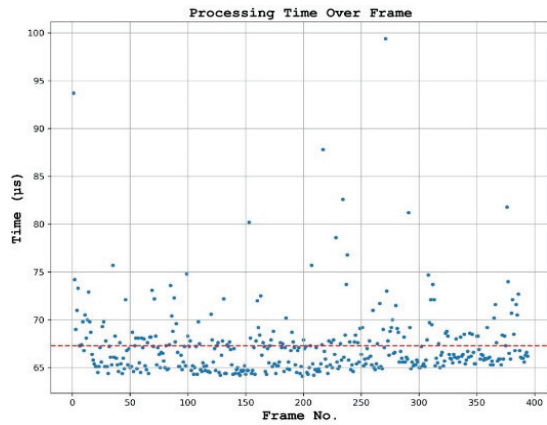
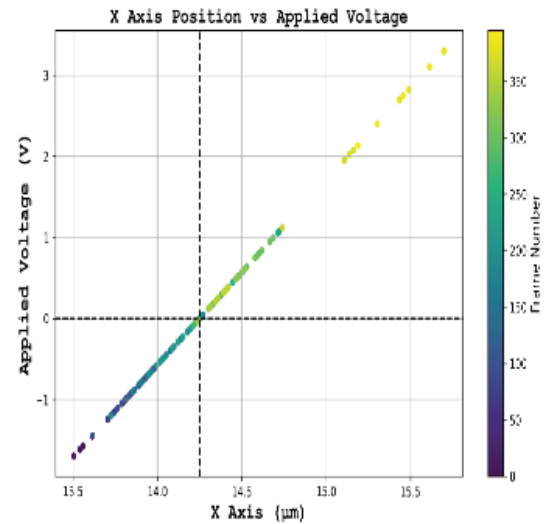
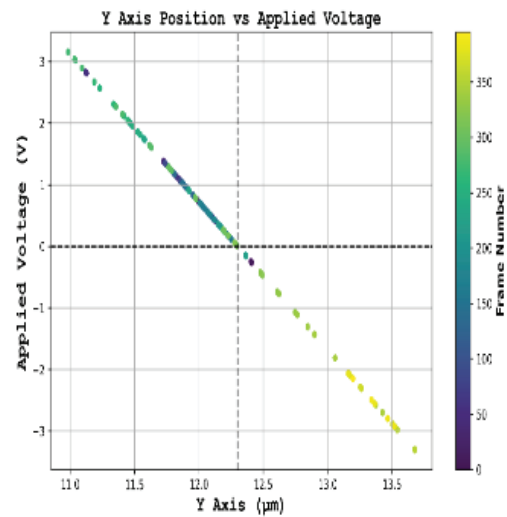


Fig. 7: Frame-by-frame processing time recorded by the image-processing algorithm for 395 video frames from Movie-3 of Kuhn et al. [16]. The latency was measured using internal timestamps, with the red dashed line representing the average duration per frame.

To assess the computational efficiency of our proposed image-processing-based ABEL trapping algorithm, we analyzed the algorithm-internal latency across 395 frames extracted from Movie-3 of the supplementary material in Kuhn et al. [16]. For each frame, the algorithm's processing duration was recorded in microseconds (μs), based on internal time-stamping mechanisms embedded within the codebase. As shown in Fig. 7, the algorithm exhibited highly consistent frame-wise processing latency. The average per-frame latency was approximately $67.5 \mu\text{s}$, with a maximum of $100 \mu\text{s}$. As shown in Fig. 7, the algorithm exhibited highly consistent frame-wise processing latency. The average per-frame latency was approximately $67.5 \mu\text{s}$, with a maximum of $100 \mu\text{s}$. Prior ABEL trap implementations reported closed-loop latencies limited by camera frame rate or fluorescence modulation cycle, approximately 3.4 ms for Cohen and Moerner [16] and $1\text{--}1.25 \text{ ms}$ for Kuhn et al. [14] and Rahman et al. [17]. The sub- $100 \mu\text{s}$ latency achieved by the proposed image-processing and feedback algorithm represents more than an order-of-magnitude reduction in processing latency compared with these reported implementations. However, since the present work was validated using published video datasets rather than a live ABEL trap, direct comparison with experimentally measured closed-loop trapping performance remains a subject of future work. However, it should be noted that the latency time is somewhat related to the size to the size of the ROI.



(a)

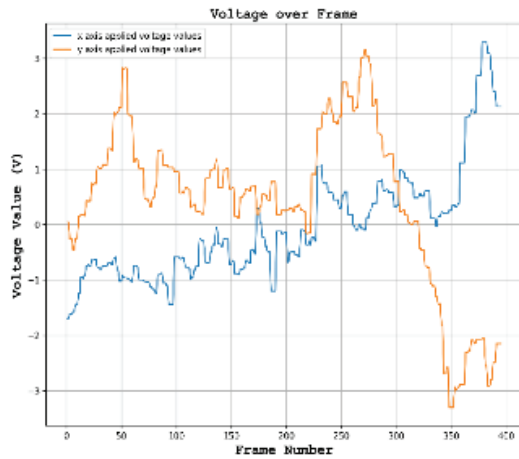


(b)

Fig. 8: Variation of the applied voltage on the two electrode axes for guiding the particle toward the trapping position. (a) Applied voltage along the x-axis electrodes. (b) Applied voltage along the y-axis electrodes.

We now present the analysis of electrokinetic feedback voltages generated by the image-based tracking system using the video dataset from Kuhn et al. [14] Fig. 8(a) shows the relationship between the particle position along the x-axis and the corresponding voltage applied to the V_x electrode. Likewise, Fig. 8(b) illustrates the particle position along the y-axis in relation to the applied V_y voltage. The control algorithm was configured to maintain a target trapping point at $14.25 \mu\text{m}$ on the x-axis and $12.30 \mu\text{m}$ on the y-axis. As the particle moved away from these target coordinates, the V_x and V_y voltages were adjusted proportionally within a $\pm 3.3 \text{ V}$ range. These

voltage changes followed the direction of displacement, demonstrating the expected linear feedback behavior and confirming the system is capable of maintaining accurate positional control.



(a)

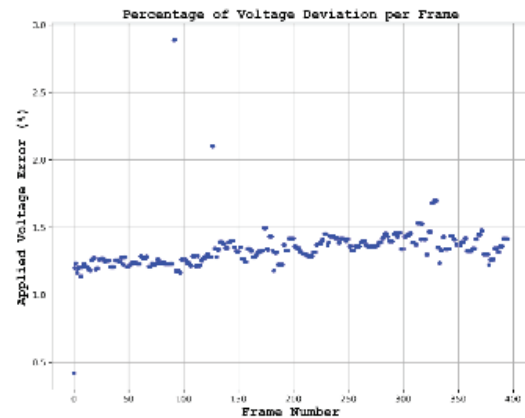


(b)

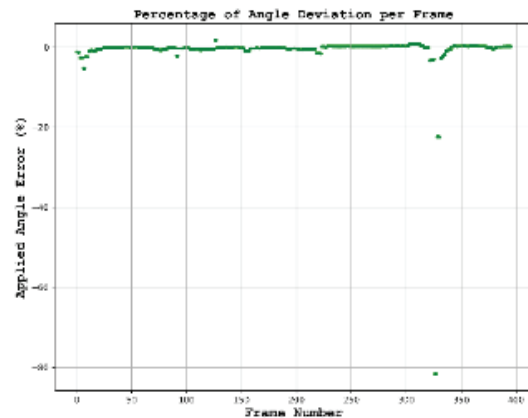
Fig. 9: Variation in the voltage waveform according to the position of particle in the recorded video by Kuhn et al. [14] (a) voltage output curves as a function of frames. (b) captured voltage waveform from the digital oscilloscope.

Furthermore, Fig. 9(a) presents the voltage output curves of V_x and V_y as a function of frame number. The yellow/orange trace corresponds to the V_y voltage, while the blue trace represents the V_x voltage. Over approximately 395 frames, both signals fluctuate within the range of -3.3 V to $+3.3$ V. Fig. 9(b) displays the voltage waveforms captured directly using a digital oscilloscope, representing the physically generated V_x and V_y signals. These waveforms closely align with the simulated voltage output

curves, with the yellow/orange trace (V_y) exhibiting synchronized modulation bursts alongside the blue trace (V_x). Additionally, the oscilloscope data indicate that processing all 395 frames required approximately 162 ms. This demonstrates the system's efficiency in real-time feedback operations, as the video was processed at 2400 frames per second. However, performance may vary depending on the trapping zone or experimental setup.



(a)



(b)

Fig. 10: Error analysis between physical setup data and simulation data: (a) resultant voltage error, (b) angle error.

Moreover, Fig. 10(a) presents the percentage error in the magnitude of the resultant voltage generated across 395 frames. The error remains within 1.5% for the majority of frames, with occasional spikes reaching up to 3%. Fig. 10(b) displays the angular error between the simulated and measured voltage vectors, where most values stay below 2%, indicating a strong agreement between the simulated outputs and the actual control signals. The occasional spikes in both magnitude and angular error occur primarily when the particle is located extremely

close to, or directly on, the reference trapping coordinate. In these instances, the required control voltages are in the low millivolt range. As a result, minor system noise may introduce relatively larger percentage deviations. However, these variations are not significant enough to disrupt the trapping performance, as the particle remains effectively within the desired region. These results confirm that the voltage control subsystem operates with high fidelity and robustness under real-time conditions.

4. CONCLUSION

In summary, we have developed an image-processing-based ABEL trapping system integrated with an efficient feedback voltage controller. The system combines real-time particle tracking with responsive electrokinetic feedback, enabling stable and consistent confinement of biomolecular targets. The proposed methodology was validated using publicly available video datasets, demonstrating effective performance across diverse particle types and trapping conditions. High agreement between simulated and measured control signals further confirms the accuracy of the system. Beyond reducing feedback latency, the proposed framework demonstrates that an image-processing-based feedback architecture can provide a practical and computationally efficient alternative for real-time ABEL trapping, offering a simpler implementation while maintaining accurate electrokinetic control. This work establishes a practical and adaptable framework for ABEL trapping, with strong potential for applications in single-molecule studies and real-time optofluidic manipulation.

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NH (Nahid Hasan) and RI (Rahul Islam) contributed to the development of the algorithm and performed the data analysis. ABS (Md Asif Bin Sayed) was responsible for data collection and visualization. SE (Sanjid E Elahi) and MR (Mahmudur Rahman) developed the conceptual foundation of the article. SCB (Swopan Chandra Barman) contributed to the overall analysis and interpretation of the results. All authors have contributed to the writing, reviewing, and commenting on the article.

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