High-bandwidth 3d force feedback optical tweezers for interactive bio-manipulation
Munan Yin, Edison Gerena, Cecile Pacoret, Sinan Haliyo, Stephane Regnier

To cite this version:

HAL Id: hal-03190970
https://hal.science/hal-03190970
Submitted on 15 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
High-bandwidth 3D Force Feedback Optical Tweezers for Interactive Bio-manipulation

Munan Yin, Edison Gerena, Cécile Pacoret, Sinan Haliyo and Stéphane Régnier

Abstract—Optical Tweezers are considered one of the most suitable techniques for biological tasks, however the lack of automation makes this technology less accessible. We present here a new 3D force sensing method with high bandwidth (up to 10KHz) which can allow implementing complex robotic approaches. Proposed technique uses high speed image tracking with nano-metric resolution in 3 directions. Its capabilities are demonstrated in a teleoperated 3D manipulation scenario with a haptic user interface, where naive users performed direct in vitro haptic exploration of isolated Red Blood Cells inside a Petri dish.

I. INTRODUCTION

Among a variety of microrobotic techniques, Optical Tweezers are considered to be one of the most suitable for biological characterization and manipulation [1], [2]. They consist on using the radiation pressure of a tightly focused laser beam to trap a micro-object in liquid solution [3]. The generated force can go up to few hundreds of pico-Newton. Although seemingly low, this is well in the range of micro-biological interactions and Optical Tweezers have successfully applied to a range of applications from sorting biological objects [4] to assembling complex microstructures [5].

An inert, bio-compatible bead is generally used as a probe to avoid laser exposure to biological objects. Additionally, in this case the effect of the trap is akin to a linear stiffness around the focal point of the beam and the force acting on the object can be obtained by measuring its motion [6]. This particularity has led to the use of Optical Tweezers for force sensing, for example in mechanical characterization of molecular interactions [7] and of the cell membrane [8].

Current robotic research on Optical Tweezers focuses on the implementation of control techniques for automation [9], [10], [11] or teleoperation [12], [13], [14]. These works consider the trapped probe as the end-effector, and the external forces acting on the probe can be used in the feedback path to close the control loop. This forces can be provided by tracking the motion of the probe under the optical microscope. Considering the force range and the trap stiffness, pico-Newton resolution is reachable. This kind of performance would make Optical Tweezers a formidable apparatus for micromanipulation in general and for biology and biochemistry in particular. Nevertheless, high dynamics effects at the microscale and the limitations of the optical microscope render most classical algorithms useless. Hence, the lack of robust 3D tracking reduces most applications to simple planar tasks. Also, the latency and low bandwidth hurts the system stability and its real-time capabilities [15].

Several methods to improve the tracking has been developed. Most commonly, a quadrant photodiode (QDP) is used to sample the position of the target at tens of kHz with nanometric precision [16]. Nonetheless, this method is vulnerable to occlusions and disturbances and works reliably only on isolated objects. An alternative is image processing through video cameras integrated into the microscope. However, state-of-art real-time visual tracking algorithms on commercial CMOS cameras can rarely exceed 60 Hz [17]. Limiting the imaging to a smaller region of interest (ROI) can accelerate processing, at the detriment of resolution and precision [18]. By combining a high-speed CMOS with tracking implemented on GPUs (Graphics Processing Unit), 3D tracking at several of kHz is reported [19]. This approach is however fairly complex as it requires special knowledge and hardware.

As an alternative to classical CMOS cameras, silicon retina sensors were proposed [20]. These “asynchronous time-based image sensor”s are frame-free and eliminate data redundancy by design. They are shown to allow the 2D tracking at an unprecedented speed in the order of tens of kilo-Hertz [21]. Because of the particularity of the image data that they provide, well-known processing techniques cannot be used. Further investigations are especially needed for real-time 3D robust tracking.

We present here a 3D motion tracking technique using an event-based algorithm taking advantage of a silicon retina sensor. It provides pico-Newton resolution and its bandwidth reaches 10 kHz. Its capabilities are demonstrated in a teleoperated 3D manipulation scenario with a haptic user interface. This kind of control scheme is very demanding and requires indeed a feedback loop at 1 kHz for stability.

II. 3D HIGH-SPEED FORCE SENSING

A. System Description

The optical scheme of the system is shown in Fig.1 (a). It’s based on an inverted microscope where the same objective is used for both imaging and producing the optical trap. Its singularity is to include an ATIS, “asynchronous time-based...
image sensor\textsuperscript{\textregistered} silicon retina. ATIS has the particularity to be adaptable to high-speed on-line tracking due to its efficient encoding of movements, as the data redundancy is eliminated by design. Each pixel is electronically independent and generate events immediately when a light variation above a threshold is detected. This data is asynchronous and an event may have positive or negative polarity. More details of this technology are discussed in [22], [23].

In a scene with the stable light environment, only the dynamic information stimulated by moving object is recorded. In the case of the presented system, the trap center has a fixed position in the ATIS image. With an appropriate threshold, image variations are mostly generated on the contour of the trapped bead as shown in Fig.1 (b). This information is exploited to infer the 3D motion of the probe.

B. 3D Tracking

Fig.1 (b) shows the conventional and event-based images of the probe. In ATIS image, accumulated events can be grouped in two concentric circles. Their center position is related to the planar motion of the probe while the radius is linked to the depth position. An event-based ring tracking algorithm is developed. It’s used to recognize both circles, their center position is linked to the depth position. An event-based ring tracking algorithm is developed. It’s used to recognize both circles, and the circle center is the region of interest (ROI) of one circle model at time \( t \) [25]. Since the fitting methods are vulnerable to noise, the ROI is used as solution to filter the outliers.

Suppose that the unknown circle model’s parameters at time \( t \) is \( C_t(\mathbf{P}_t, R_t) \), where \( \mathbf{P}_t = (X_t, Y_t) \) is the circle center’s position and \( R_t \) is the radius. Then, a fast non-iterative algebraic fit [26] minimizes the cost function:

\[
\min_{\mathbf{P}_t \in \mathbb{R}^2, R_t \in \mathbb{R}} \sum_{\mathbf{p}_k \in U(t)} ||d(\mathbf{p}_k, \mathbf{P}_t)^2 - R_t^2||^2
\]

where \( \mathbf{p}_k \) is the \( k \)th event’s location in \( U(t) \). \( d(\mathbf{p}_k, \mathbf{P}_t) \) is Euclidean distance between \( \mathbf{p}_k \) and the circle center \( \mathbf{P}_t \). Introducing parameters \( A = -2X_t \), \( B = -2Y_t \), and \( C = X_t^2 + Y_t^2 - R_t^2 \), (2) can be written as a linear least square problem:

\[
\min_{\mathbf{P}_t \in \mathbb{R}^2, R_t \in \mathbb{R}} \sum_{\mathbf{p}_k \in U(t)} ||Ax_k + By_k + C + x_k^2 + y_k^2||^2
\]

Solving A, B, and C, gives the parameters of circle \( (\mathbf{P}_t, R_t) \). This single circle tracking method is used to parametrize the two concentric circles of the ring (inner \( C^1_t \) and outer \( C^2_t \)). All incoming event occurring in a considered time interval are assigned to the closest circle model. Events at the intersection of two ROIs are discarded to reduce the ambiguity. This condition makes sure that the inner and outer circle will not become into one. The ROIs are updated accordingly. The algorithm is given below.

\textbf{Algorithm 1} Event-based Robust Ring Fitting

\begin{algorithm}
\REQUIRE{Events $E(p,t)$}
\FOR{every step do}
\STATE Update the content of $U^1(t)$ and $U^2(t)$ according to (1)
\STATE Estimate $C^1_t$ and $C^2_t$ parameters according to (3).
\STATE Update output : $[X^1_{t+1}, Y^1_{t+1}, R^1_{t+1}]$.
\STATE Update $ROI^1_{t+1}$ and $ROI^2_{t+1}$.
\ENDFOR
\end{algorithm}
C. Evaluation of tracking

1) Range and resolution: A 3 µm silica microsphere fixed to the sample-holder is used to evaluate the tracking. Fig.2 gives the real displacement, as reported by the nanostage vs tracked motion in pixels for all directions as well as the calculated pixel/µm transform.

The detectable motion range is 6µm and 7µm for x and y respectively with less than 3% standard deviation (SD). On z-axis, the relative radii variance is shown in Fig.2 (c). The linear detection range is about ±2 µm around the focus plane with 5 % SD. This 3D detection range (6 × 7 × 4µm) is sufficient since the linearity of the trap stiffness is valid around one diameter of the trapped bead [6], here 3 × 3 × 3µm³.

With 204×304 pixels, the theoretical resolution is 23.8nm/pixel in x and y and 166.6 nm/pixel in z. Practically, the bead center position and radius were estimated with sub-pixel accuracy using the circle tracking algorithm. This sensitivity varies in different illumination conditions and working environment.

2) Robustness: Robustness here is defined as the ability to extract the 3D position of the target from noisy data, or even a small subset of data. It will be tested under two most commonly encountered situations partial occlusion i.e. only part of the target image is captured, and obstacles disturbances i.e. to track the target among many obstacles.

In the occlusion test, the microsphere’s image is partially out of the view of ATIS. The tracking errors for 30% occlusion of the inner circle are shown in Fig.3(c). As can be seen, for less than 30% occlusion, the tracking error and the SD are less than 5% for both lateral and axial detection.

In obstacles disturbance test, the target bead (in the center) is surrounded by two other similar objects as shown in Fig.3 (d). They are fixed on Petri-dish and animated with a sinusoidal movement. The tracking result is shown in Fig.3 (f). The lateral tracking errors are less than ±5% with 2% SD in within ±2.5 µm. The axial errors are less than ±20% around the focus plane with less than 10% SD. As the image plane move far away from focus plane, the radius error increases up to 30%.

3) Computational Load: The algorithm is implemented in C++ on a hard real-time framework. The testing relies on a 2.9 GHz Dual core desktop PC, with a total CPU load about 50 % of its power and a memory consumption of about 4 MB. The average running time for each iteration is less than 2.9 µs, with less than 2 µs deviation. Then the system is successfully pushed to 10 kHz real-time sampling rate.

III. 3D HAPTIC FEEDBACK OPTICAL TWEEZERS

A. Haptic Coupling

In order to show its benefits and validate the proposed force sensing, a teleopration scenario with haptic feedback is implemented. This is a demanding application from the control point of view as the stability and transparency of haptic feedback coupling requires a sampling of 1 kHz [27], [15]. Previous works presented haptic feedback on Optical
Tweezers but were limited to 2D [13], [14]. The 3D tracking allows for a real spatial coupling in this case.

Haptic feedback requires a bilateral control scheme, as presented in Fig. 4. In this scheme, the user handles the haptic interface (Omega.7, ForceDimension). Its position is used to control the motion of the sample through the nano-stage. The force measured on the probe is feedback to the user with an appropriate gain.

Motion of the master device is scaled down by $6 \times 10^{-4}$ to drive the trap position relatively to sample holder. Actually, the trap position is fixed, and the mobile part is the nano-stage holding the samples. Nano-stage can be driven in two modes, rate-control to cover the whole working space (200×200 µm), and position-control to execute precise tasks. The measured forces are magnified by $1 \times 10^{12}$ and sent back to the user. The force on the trap is calculated using the optical force model [6]:

$$F_{opt} = K \times (P_{laser} - P_{probe})$$  \hspace{1cm} (4)

where $P_{laser} - P_{probe}$ represent the displacement between the laser and the probe position as obtained from the tracking method. $K$ is the stiffness of the trap. This stiffness can be calculated experimentally using the Equipartition method [28]. Considering 30 mW laser power it is estimated in x-axis, y-axis and z-axis as $K_x=12.3$ pN/µm, $K_y=12.6$ pN/µm, and $K_z=1.5$ pN/µm respectively under room temperature of 25.5 °C.

A single PC (Intel Xeon core, 2.93 GHz) operating under a real-time co-kernel Linux and RTOS APIs Xenomai is used to control the system. The control-loop runs with a force refresh rate of 1 kHz.

<table>
<thead>
<tr>
<th>Table I: Summary of System Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTs stiffness (pN/µm)</td>
</tr>
<tr>
<td>Force detection range (pN)</td>
</tr>
<tr>
<td>Force resolution (pN)</td>
</tr>
<tr>
<td>Haptic loop</td>
</tr>
</tbody>
</table>

B. 3D Haptic Experiments on biologic samples

Biologic samples are chosen to illustrate the use of the system in a real world scenario. Red Blood Cells (RBC) are easy to acquire and have an 3D irregular dumbbell-shaped profile, and hence are well suited for this illustration. They are fixed in 4% formaldehyde for biological stability. Probes are 3µm polystyrene beads incubated in PBS and Ethylenediaminetetraacetic acid (EDTA) solution to prevent the surface sticking.

1) z-axis Haptic Feedback: The first experiment will validate the force feedback on z axis. The RBC sample is fixed on the bottom of a Petri Dish. The user first traps a bead to serve as probe, then moves the sample-holder as to position the probe above a fixed RBC. The planar motion of the nano-stage is then artificially blocked and the user controls only the z motion until the probe touches the cell and he feels an obvious counter-force. The experimental results are shown in Figure 5.A. Small fluctuations are caused by the 3D Brownian motion of probe which are largely present at the considered scale. The contact point is shown in region II in (a), (b), (c). At contact, a sudden reaction force in the z-axis of about 0.3 pN is detected, and the Brownian motion is attenuated compared to the region I. Then the probe is pushed deeper into the cell until 1.6 pN in the z-direction, as shown in III. At this time, user felt about 1.6 N force in the z-axis. The cell is contacted twice during the presented experiment. Similar results are obtained during the two passes which also proves the repeatability of the axial force detection.

Notice that this experiment aims to validate the axial haptic feedback during biological manipulations. In addition, the stiffness of the RBCs can be roughly obtained from the result. By using the Hertz model, which considers the cell a homogeneous smooth semi-sphere, the elastic modulus of the RBC is calculated as 33.7 Pa [29]. This result is similar to literature, with the measure of the elastic modulus of adherent living cells [30]. This is an approximated result; for a proper mechanical characterization, the trap stiffness should be calibrated in the neighborhood of the cell before [31]. The system would also allow to automatize the experiment and to repeat it accurately over a large number of trials.

2) 3D Haptic Exploration: This experiment is dedicated to touch the 3D contour of cells and explore their shapes. The difficulty of these tasks comes from the uncertainty and irregularity of the biological objects. Since the visual information may be blurry or lost at some parts, the haptic feedback will help users to maintain the contact and decrease the possibility of losing the trap.

This experiment is dedicated to let a user touch the 3D contour of cells and explore their shapes. The difficulty of these tasks comes from the uncertainty and irregularity of biological objects. Since the visual information is blurry and lacks the depth of field, and the probe is eventually occluded by the sample itself, the haptic feedback would help to maintain the contact.

First, 2 flat and transparent RBCs, damaged after the hemoglobin leakage, stuck together forming a ∞ shape is explored (fig. 5.B). Another experiment is conducted on a isolated RBC (fig. 5.C). The 3D contact force is successfully perceived and maintained by operators. Haptic feedback allows to keep the contact even when the probe is occluded and users were able to achieve a surface exploration with consistent force feedback.
3) Preliminary user evaluations: To further prove the repeatability and effectiveness of the 3D haptic feedback during biological manipulations, a preliminary users study is conducted. 6 volunteers explored the shape of one identical RBC. Each were subject to those three different conditions: with only vision feedback, both vision and haptic feedback, and only haptic feedback respectively. Before the formal evaluation, the participants were trained to use the system for about 10 minutes and shown how to trap microbeads to serve as probes. They were than asked to roll the probe around the cell while keeping the contact at all times. The task under each condition was conducted twice. The probe trajectory during three users’ experiments are depicted in Fig. 6 (a), (b) and (c).

During this experiment, it is observed that exploration with only haptic information is time-consuming. Users are required more to concentrate on the force feedback hence move slowly. Full exploration is successfully completed only in half of the trials. Due to the lack of vision information, the users can hardly estimate the path to follow. With only vision feedback, users frequently lose the trapped object (33%) because of large contact forces difficult to infer from vision alone. This is also probably caused by some shadows and invisible features that are barely noticed.

Combining the haptic feedback and vision overcomes the above shortages. Vision provides general overview information of the scene and haptic feedback allows for fine control skills. The haptic feedback largely decreases the rate of losing trap to 17% and users performs the task more efficiently. Note however that this is a preliminary qualitative analysis. A formal user evaluation comparing both modalities is planned.

IV. CONCLUSION AND FUTURE WORK

We have presented a new method for 3D force sensing for Optical Tweezers, with high bandwidth (up to 10Khz), in a large three-dimensional workspace that covers the full linear domain of a single trapped bead and pico-newton sensibility with a theoretical resolution of 0.3pN. The force sensing is based on high-speed optical processing implemented through a silicon retina and its dedicated tracking algorithm.
Thanks to the capabilities of force sensing, and because of the stable and simple set-up design, complex tasks have been demonstrated in real biological environment on a haptic teleoperation platform. This is a good illustration of the kind of new robotic application that can benefit from the proposed 3D sensing. A simple user study has shown that non-expert user can take in hand the optical tweezers after few minutes of training. It is safe to assume that expert user will be able to perform complex tasks more efficiently.

The real-time particle-tracking scheme can be also extended to magnetic tweezers and other micro-robotics techniques. The method remains compatible with fluorescence, interferential and superresolution microscopy. An interesting development would be a Scanning Force Microscopy technique adapted to biological environments.

In future works, we plan to extend this fast 3D force sensing capabilities to several trapped objects. Current state-of-the-art of multi-trap optical tweezers usually do not include force sensing capabilities [32]. Multi-trap optical tweezers or optically actuated microtools [33] with highly transparent force feedback will be advantageous for numerous biomedical applications.

**References**


