Biomicrofluidics

RESEARCH ARTICLE | DECEMBER 04 2023

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Biomicrofluidics 17, 061302 (2023)
https://doi.org/10.1063/5.0174269

Biomicrofluidics PERSPECTIVE pubs.aip.org/aip/bmf

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Cite as: Biomicrofluidics 17, 061302 (2023); doi: 10.1063/5.0174269
Submitted: 29 August 2023 · Accepted: 16 November 2023 ·
Published Online: 4 December 2023

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ABSTRACT

Particle image velocimetry and particle tracking velocimetry have played pivotal roles in flow and particle characterization, owing to their non-invasive and accurate data collection methods. However, their broader application in the biomicrofluidics field is constrained by challenges, such as intensive calibration, high post-processing costs, and optical compatibility issues, especially in settings where space is a bottleneck. This article describes recent advancements in non-iterative ray tracing that promise more streamlined post-capture calibration and highlights examples of applications and areas that merit further technological investigation. The development and adoption of these techniques may pave the way for new innovations.

I. INTRODUCTION

In the realm of fluid mechanics, particularly within the biomicrofluidics area, progress may be significantly accelerated with advancements in image-based 3D reconstruction methodologies. Particle Image Velocimetry (PIV) and Particle Tracking Velocimetry (PTV) and variants offer solid foundations. Their prominence within the scientific community is given by their non-invasive capabilities, accuracy, and adaptability. However, inherent challenges associated with calibration complexity, extensive post-processing, and optical compatibility underscore the need for further innovative solutions to optimize these methodologies.

Advancements in microlens technology and high-resolution camera sensors have led to the emergence of innovative techniques for flow characterization and particle tracking using a singular light field (LF) camera.[1–6] This approach is especially suitable for biomicrofluidics, presenting numerous avenues for technological refinement. Enhancements in optics, computational vision, mathematical modeling, parallel computing, and artificial intelligence are paving the way for advanced real-time diagnostic systems tailored for general configurations.

In particular, integrating deep learning and artificial intelligence is expected to particularly contribute in the context of 3D tracking. Leveraging datasets PIV and PTV, neural networks can optimize and refine the 3D particle tracking. Traditional calibration methods, often time-consuming and prone to error from various sources, can be streamlined with the assistance of these networks. With the predictive power of artificial intelligence, it becomes feasible to foresee and improve calibration challenges in real time, enabling a more efficient and accurate tracking process.

Possibilities are vast and may reach new horizons. For instance, as our understanding of molecular dynamics grows, it is possible to have real-time 3D tracking of DNA transcription using fluorescent fusions, elevating our grasp on processes at the core of life.[7,8] Such advancements would uncover DNA mechanisms and could speed up progress in drug development, genetic modification, and our knowledge of diseases. The potential for these 3D reconstructions will extend to multi-cellular interactions during crucial biological events. Visualizing, characterizing, and quantifying in 3D how cells behave during tissue regeneration or in the onset of diseases can be a game-changer, furthering our knowledge and refining therapeutic strategies.[9,10] As the convergence of technology continues, 3D bioprinting has a special place. With real-time feedback from advanced image-based 3D reconstructions, the production of functional tissues, organs, or complex cellular formations could be expedited.[11]

Although existing biomicrofluidics tools have led to significant progress, integrating new technologies hints at a future filled with untapped potential and increased accuracy.

II. TECHNIQUES

Illumination constraints and space limitations frequently hinder traditional 3D photogrammetry methods, especially in small-scale biological experiments. To address these challenges, alternative methodologies have surfaced in recent years. These encompass holographic,[12–15] confocal,[16,17] stereoscopic,[18,19] defocus-based,[20,21] active scanning,[22,23] and structured volume illumination techniques.[24] The evolution in microlens production, paired with innovations in camera sensor technology, has fostered the emergence of light field (LF) technology in 3D photogrammetry.[25] Also, LF photogrammetry has seen integration with other methods, such as PIV[1] for three-dimensional flow measurements. Unlike other techniques that require customized illumination setups, complex components, or synchronized operations between cameras and specific illumination sources, LF 3D photogrammetry has unique advantages. It is pre-calibrated, exhibits versatility, and is cost-efficient, aligning with a wide spectrum of optical configurations.

Despite its potential, the LF approach has not been widely adopted for 3D reconstruction in the micro-biological realm, due to challenges in implementation and operation. Traditional post-processing techniques associated with this method can be computationally intensive. Also, an absence of a clear, standardized protocol, may make it not advantageous. This complexity arises as these methods require adjustments tailored to the specific computational capacities at hand.

At its core, the 3D reconstruction process aims to recover the 3D intensity distribution $E(x, y, z)$,

$$\sum_{j\in N_i} w_{i,j} E(x_j, y_j, z_j) = I(x_i, y_i). \quad (1)$$

Here, N_i represents the count of voxels that fall within the line-of-sight of the ith pixel. The coefficient $w_{i,j}$ is a weighting factor, describing the relationship between the 3D voxels and their 2D pixel counterparts and $I(x, y)$ denotes a 2D image. One of the prevalent methods to tackle this inherently complex problem is the Multiplicative Algebraic Reconstruction Technique (MART). Belden *et al.*[26] pioneered this approach, which was initially used in computed tomography (CT) to reconstruct 3D surfaces from a series of angular projections due to its strong performance in background reconstruction and boundary handling. MART uses these 2D angular projections and correspondence information, iteratively decoding them to obtain 3D structural information as follows:

$$E(x_j, y_j, z_j)^{k+1} = E(x_j, y_j, z_j)^k \left(\frac{I(x_i, y_i)}{\sum_{j\in N_j} w_{i,j} E(x_j, y_j, z_j)^k} \right)^{\mu w_{i,j}}. \quad (2)$$

Owing to the considerable data size of $w_{i,j}$ (may exceed 100 GB), the process of iterative solving proves to be laborious. This remains true even when leveraging optimized tile loading via a RAID 0 solid-state disk configuration. Also, precise estimation of the parameter w, requiring pixel-level accuracy in the calibration phase, introduces an additional challenge. This complexity is a barrier to the broader application of LF photogrammetry in practical scenarios.

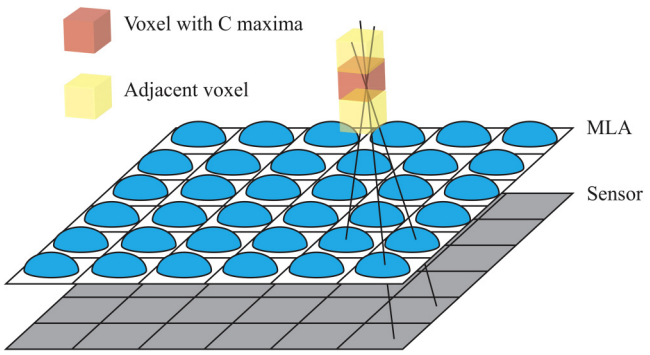

FIG. 1. Basic schematics of a 3D light ray diagram of an image volume around the microlens array (MLA).

To expedite computation, the MART method has been enhanced through the incorporation of dense ray tracing-based MART[2] and the pre-recognition simultaneous algebraic reconstruction technique (PR-SART),[27] which exhibits greater resilience to noise and quicker convergence. Levoy *et al.*[25] and Truscott *et al.*[4] used a 3D deconvolution algorithm based on an estimated point-spread-function (PSF) approach. Compared to algebraic techniques, this 3D deconvolution is more efficient, as it avoids the need to store and manipulate extensive weighting matrices.

Within this framework, we introduced a non-iterative ray tracing technique, complemented by a robust post-capture calibration process; see specific details described in Hong and Chamorro.[6] Unlike traditional methods of 3D intensity retrieval, our proposed approach streamlines the process, achieving near-instantaneous reconstruction. This is made possible by efficiently managing sparse cloud points generated via the ray counter matrix, as illustrated in Fig. 1. Consequently, the exhaustive computations conventionally associated with the weight matrix w and iterative procedures are no longer necessary. In our approach, ray tracing hinges on the two-point method, which uses pixels and their corresponding lenslet centers. Remarkably, this entire process is amenable to parallelization on a GPU, allowing for a fast reconstruction speed of roughly 100 ms for each frame.

III. IMPLICATIONS AND APPLICATIONS

The discussed framework holds particular promise for probing the motion and dynamics of micro-organisms,[28] an area of paramount importance in biotechnological and environmental areas. The capability of real-time reconstruction and tracking allows for visualizing, and analyzing complex swimming patterns, trajectory shifts, collective dispersion, and accelerations in reaction to various triggers or environmental changes. Examples include behavior exhibited by, e.g., phototrophs and chemotrophs.[29]

Optoelectronic tweezers (OET)[30,31] is a valuable non-contact micromanipulation technology, adept at governing the behavior of microparticles and cells.[32] The main advantage of OET is its accuracy, allowing the efficient handling of particles, cells, and other small entities without direct physical interaction or the intervention

of mechanical apparatuses. Incorporating real-time 3D tracking further augments their efficacy.

Microrobot swarms may offer precise delivery mechanisms. A micro-scale robot, operating within a group, can be directed to specific tasks or locations, making it a critical tool for regulated operations. The ability to track three-dimensional positions in real-time amplifies the potential of microrobots in biological microfluid environments. Such real-time tracking is pivotal when these microrobots are exposed to external forces; they include magnetic pulls, minor acoustic wave pressure, and electric fields. Knowing microrobot trajectories may also inform instantaneous adjustments, refining their motion patterns or strategies in real time. Real-time 3D tracking can provide immediate feedback in a robot that deviates from its intended course, allowing timely corrective measures. Wang and Zhang[33] explored the dynamics of such systems, highlighting the benefits and the challenges that remain in harnessing the full potential of microrobot swarms.

Microfluidic devices have emerged at the forefront of innovative biomedical applications, pushing the boundaries of precision medicine and personalized therapies. When considering their potential in fields, such as the production of therapeutic proteins,[34] cultivating cells in a 3D environment,[35] and developing advanced systems for drug delivery,[36] the breadth and depth of their utility are clear. At the heart of maximizing the potential of these devices is the understanding and control of fluid dynamics within their networks. This allows for understanding flow patterns and how drug particles, RNA, DNA, and other key players behave within the confined, controlled spaces of microchannels.

Employing microfluidic technologies in tandem with real-time tracking and management augments the potential for refined precision in both biomedical research and therapeutic pursuits, among others. For example, real-time insight into mixing efficiency can facilitate optimal reactions and interactions.

IV. CONCLUSIONS AND UPCOMING HORIZONS

The integration of ray tracing techniques with the bio-microfluidics area signals a paradigm shift in biomechanics and fluid research. By addressing the inherent challenges faced in traditional Particle Image Velocimetry (PIV) and Particle Tracking Velocimetry (PTV) approaches, the approach discussed streamlines research processes and opens avenues for a deeper, and broad range of investigations.

The ability to provide real-time, precise 3D data expedites research and potentially enables new applications. The applications are multifaceted and transformative, from understanding microorganism behavior to refining the precision and efficiency of optoelectronic tweezers. In the realm of microrobotics, real-time 3D positioning paves the way for enhancing control and maneuverability. Such advancements can greatly influence fields from medicine to environmental research, showcasing the vast potential of synergies. The implications for microfluidic device design are vast. As real-time particle tracking unveils in-depth insights into fluid dynamics, it allows for more efficient, adaptive, and versatile microfluidic systems. Such enhancements have far-reaching implications in drug delivery, therapeutic protein production, and even 3D cell cultures with the possibility of active control and adaptation when needed.

Finally, it is worth stressing that ray tracing's integration into bio-microfluidic systems may shape the trajectory of biomicrofluidic research.

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Liu Hong: Conceptualization (equal); Formal analysis (lead); Methodology (equal); Software (lead); Writing – original draft (equal); Writing – review & editing (supporting). **Leonardo P. Chamorro:** Conceptualization (equal); Funding acquisition (lead); Methodology (equal); Supervision (lead); Writing – original draft (equal); Writing – review & editing (lead).

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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