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Design of Droplet Microfluidic Sorting and Counting System based on Object Detection and Tracking Algorithm

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ABSTRACT

Droplet microfluidics, which encapsulates individual cells within separate microreactors, has become an essential tool for single-cell phenotypic and genotypic analysis. However, the efficiency of single-cell encapsulation is limited by the Poisson distribution governing the encapsulation process, resulting in most droplets being either empty or containing multiple cells. Traditional single-cell sorting methods typically rely on fluorescence labeling for identification, but this approach not only increases experimental costs and complexity but can also impact cell viability. Additionally, current label-free sorting methods still encounter difficulties in accurately detecting multicellular droplets and small cellular aggregates. To address these challenges, this paper proposes an intelligent sorting system that combines YOLOv8 object detection and BoTSORT tracking algorithms. This system enables real-time analysis of droplet images, facilitating precise identification, counting, and automated sorting of target droplets. To validate the system's performance, polystyrene microspheres were used to simulate real cells in sorting tests. The results demonstrated that, under label-free conditions, the system significantly outperformed traditional fluorescence labeling methods in both classification accuracy and sorting efficiency. This system provides an effective, label-free solution for cell sorting, with potential applications in precision medicine, single-cell sequencing, and drug screening.

1. Introduction

With the rapid development of single-cell analysis technologies, droplet microfluidics has become a vital tool for examining the phenotypic and genotypic characteristics of individual cells. By encapsulating individual cells within microdroplets, this technology creates a biomimetic microenvironment that enables precise biological and molecular analyses in a controlled setting(Gardner et al., 2022). Its emergence has provided robust support for a range of biomedical applications, including cell therapy, early disease diagnosis, and drug screening.

However, despite its many advantages, droplet microfluidics still faces significant technical challenges. One major issue is the phenomenon of multicell co-encapsulation, which greatly reduces the efficiency and reliability of single-cell analyses. Due to the Poisson distribution

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that governs the encapsulation process, only about 36% of the droplets contain a single cell, while the majority are either empty or contain multiple cells(Link et al., 2022). This results in a biological throughput that is roughly three times lower than the physical throughput, limiting the practical effectiveness of the technology. Moreover, the co-encapsulation of multiple cells compromises the precision of single-cell analysis and hinders applications such as single-cell sequencing, drug screening, and cell therapy(Zhong et al., 2023). Therefore, improving the purity of single-cell encapsulation and preventing multi-cell co-encapsulation have become key challenges in advancing droplet microfluidic technology.

Currently, droplet microfluidic sorting techniques can be broadly classified into passive and active methods. Passive sorting relies on the inherent channel geometry and hydrodynamics, such as deterministic lateral displacement (DLD)(Hochstetter et al., 2020) and inertial microfluidics(Kemna et al., 2012) to separate cells. Although these methods are straightforward and offer high throughput, they lack real-time controllability and flexibility, and their accuracy is often insufficient for complex applications. In contrast, active sorting employs external force fields, such as electric, magnetic, acoustic, pneumatic, or thermal to precisely control and sort droplets in real time(Xi et al., 2017). However, active sorting methods typically depend on fluorescence labeling for cell identification. While fluorescence labeling can achieve high recognition accuracy, it also has notable drawbacks: it may alter the intrinsic properties of cells and can introduce issues related to cytotoxicity and biocompatibility. Recently, image-based, label-free sorting methods have been developed for live cell analysis Advances in deep learning and machine learning have further opened new avenues for non-invasive, label-free cell sorting based on image analysis. Deep learning algorithms, particularly convolutional neural networks (CNNs), have shown superior performance in image analysis and feature extraction(Tang et al., 2023), enabling the automatic extraction of morphological features from cells. This capability allows for the accurate identification and sorting of single cells and multicellular aggregates in droplets. When integrated into microfluidic systems, these techniques facilitate real-time analysis of droplet contents, thereby improving sorting accuracy and throughput. For instance, Lewis Howell et al. developed a label-free sorting system with feedback control using YOLOV4-tiny. This system automatically adjusts the flow rate based on detection accuracy and counting performance, thereby regulating the loading of microbeads(Howell et al., 2021). Despite these advances, current image-based label-free sorting systems still face challenges. Most existing systems rely on complex optical setups, which can reduce system robustness and increase costs. Additionally, their detection accuracy often falls short of the demands of high-precision applications.

To address these issues, this study presents an intelligent sorting system that integrates YOLOv8 object detection with BoTSORT tracking algorithms to provide real-time statistical analysis, accurate droplet classification, and dynamic tracking. This system not only simplifies the experimental setup but also significantly enhances sorting accuracy. Compared with traditional cell labeling methods, our approach maintains high detection accuracy without the interference of cell labeling, offering greater flexibility and broader application potential. The modular design of our system allows it to be readily adapted for cutting-edge fields such as precision medicine research, single-cell sequencing, and cell therapy, thereby providing essential technical support and a new research platform for these areas.

2. Materials and methods

2.1 Microfluidic chip design

In this experiment, a flow-focusing structure is employed to generate droplets. The design of the droplet microfluidic sorting chip is created using CAD software, as shown in Figure 1. The chip features a channel depth of 80 microns, with a channel width of 80 microns before droplet formation and 120 microns after droplet generation. The chip structure includes two oil-phase inlets (labeled “Oil” in the figure), one Polystyrene-phase inlet (labeled “PS”), two collection outlets (labeled “Sorted” and “Waste”), and sorting electrodes (labeled “Electrode”) that generate dielectrophoretic forces. Figure 1a illustrates the flow-focusing structure designed to generate droplets encapsulating cells, Figure 1b shows the droplet sorting region, and Figure 1c displays the two collection channels. Surrounding the chip are 4MKCL saline grounding electrodes, which create the necessary electric field gradient for dielectrophoretic deflection. These electrodes also help limit stray electric fields that could cause droplets to accidentally merge within the channel. Once the chip design is finalized, the mask template is sent to Qingyi Optoelectronics in Shenzhen, China, for fabrication.

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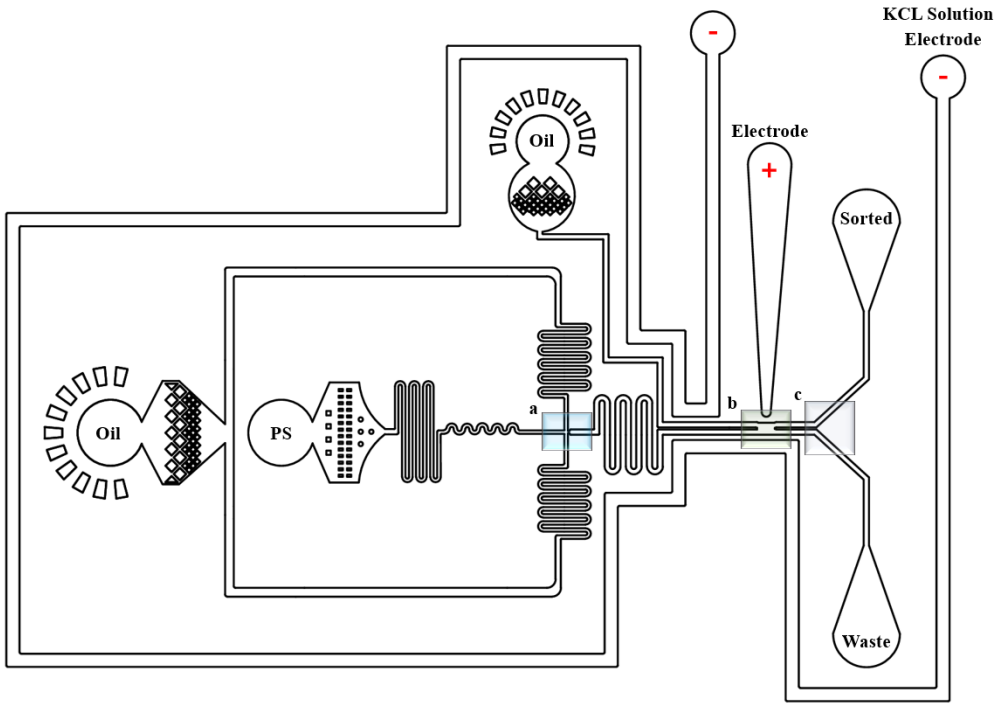

Figure 1. Structure of microfluidic sorting chip.

2.2 Microfluidic chip fabrication

The microfluidic chip is fabricated using a photolithographic master mold. To create the mold, SU-8 3050 photoresist (Microchem) is spin-coated onto a 3-inch silicon wafer. The spin speed of SU-8 3050 is adjusted based on the desired channel height for the microfluidic chip. Initially, the photoresist is spin-coated onto the wafer at 1700 rpm to achieve a thickness of approximately 80 microns. After pre-baking the wafer at 95°C for 15 minutes, a plastic photomask is placed on top, and the wafer is exposed to 25 mW UV light (PR160L, Kessil) for 30 seconds. Following exposure, the wafer undergoes a post-exposure bake at 95°C for 5 minutes. The wafer is then developed in SU-8 developer (MicroChem) for around 15 minutes, after which the master mold is cleaned with isopropanol and ethanol, and dried with nitrogen gas. Next, PDMS (SYLGARD 184, Dow Corning) is mixed with a curing agent in a 10:1 weight ratio and poured over the master mold. The PDMS is cured overnight at 70°C in an oven. Once cured, the PDMS layer is peeled off the mold, and a 0.7 mm hole punch is used to create the inlets and outlets. The PDMS layer is then bonded to a clean glass slide using oxygen plasma treatment (Beijing Saiaote, YZD08-2C). Finally, the chip is heated at 85°C for 45 minutes to improve the bonding strength. The chip fabrication process is illustrated in Figure 2a, and the finished chip is shown in Figure 2b.

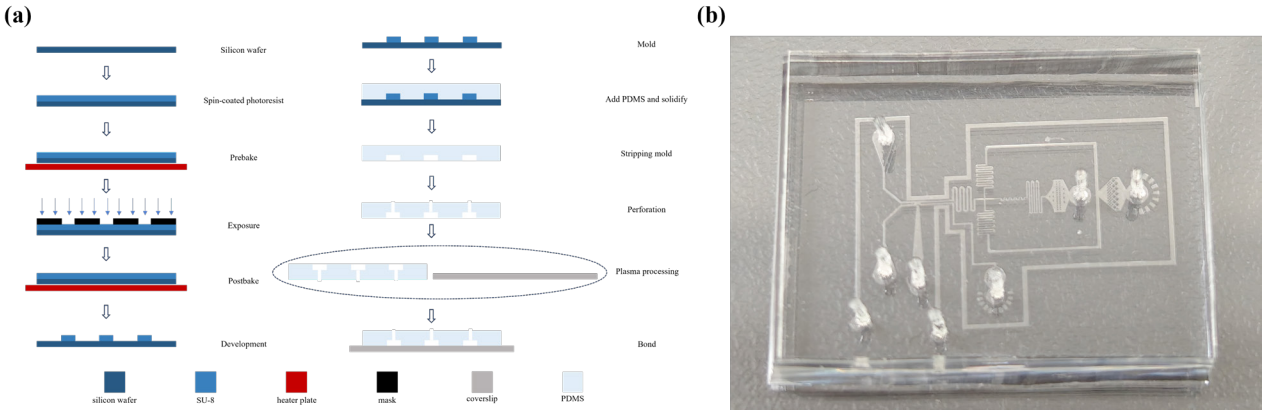

Figure 2. (a) Microfluidic chip fabrication process; (b) The chip object.

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2.3 DEP Control Module Design

This study employs dielectrophoresis (DEP) technology, whereby droplets in a liquid flow experience a force in a high-voltage AC electric field that causes them to shift toward one side. Figure 3 illustrates the DEP sorting control unit, which is composed of two main modules. The first module, the waveform generation unit, includes an FPGA control program (signal generation) and an AD9708 driver board (AD/DA conversion). The second module is the waveform amplification unit, which primarily consists of a voltage amplifier. The FPGA main program controls the sorting pulse signal generation by sending waveform control instructions to the AD9708. These instructions are transmitted between the modules to the AD9708's internal register, prompting it to output a sinusoidal signal at a predetermined frequency and duration. This signal is then amplified externally into a high-voltage AC signal and transmitted via wires to the electrodes on the droplet microfluidic sorting chip, thereby applying the necessary electric field in the sorting region to direct the droplets.

2.4 Sorting and counting algorithm based on object detection and tracking

2.4.1 Data set collection and preparation

The image dataset was captured using an inverted CCD camera. HF-7500 fluorinated oil and a prepared suspension of polystyrene microspheres were introduced into the chip, where the designed flow-focusing structure reliably generated droplets encapsulating the microspheres. The inverted CCD camera then captured real-time images of the droplets, resulting in raw images with a resolution of 1280×800 pixels. These images were manually selected and cropped to a size of 544×544 pixels. The images were then manually classified into four categories: (1) "0cell" for empty droplets, (2) "1cell" for droplets containing one polystyrene microsphere, (3) "2cell" for droplets containing two polystyrene microspheres, and (4) "3cell" for droplets containing three or more polystyrene microspheres.

2.4.2 Training of the YOLO object detection model

After completing the dataset annotations, we partitioned it into training (80%), validation (10%), and test (10%) subsets. The YOLOv8 neural network model(-Glenn et al., 2023) was trained and tested on a Windows 11 system equipped with a 64-bit operating system, an Intel i5-12500h 2.5 GHz processor, 16 GB RAM, and a CUDA-enabled NVIDIA GeForce 3050ti laptop GPU. Training the droplet classification model using YOLOv8 took approximately 25 minutes. After 231 epochs, the model's performance plateaued, achieving convergence with a mean Average Precision (mAP) of 98.8%, indicating excellent performance. Upon completion, we saved both the best-performing and the final training weights for future deployment applications.

2.4.3 BoTSORT object tracking algorithm

BoTSORT is a deep learning-based multi-object tracking algorithm(Aharon et al., 2022) designed to track detected objects across multiple consecutive frames. It takes the detection outputs from YOLOv8 as input, and first uses a Kalman filter to predict the next position of each target in the following frame. Then, a data association algorithm, which combines motion information and appearance features, is applied to match detections in the current frame with previous target trajectories. Once a match is made, BoTSORT updates the target's trajectory and assigns it a unique ID, enabling accurate cross-frame tracking. This approach ensures that each target maintains a consistent identifier throughout the video sequence, preventing duplicate counting or target loss. By integrating YOLOv8 object detection with BoTSORT tracking, the system can accurately classify and dynamically track droplets encapsulating microspheres.

3. Results and discussion

3.1 Principle of sorting and counting system

Figure 3 illustrates the label-free cell sorting and counting system that integrates object detection with tracking. In this system, the deep learning-based YOLOv8 object detection algorithm and the BoTSORT tracking algorithm are used to perform real-time detection, classification, and counting of polystyrene microspheres encapsulated in water-in-oil droplets captured by bright-field imaging. As the droplets containing polystyrene microspheres pass through the chip's sorting region, a CCD camera continuously acquires images at 60 fps, which are then transmitted to a computer. There, the trained YOLOv8 network processes each frame to determine the droplet type and position. The detection results from multiple frames are subsequently correlated using the BoTSORT tracking algorithm, which assigns a unique ID to each droplet for dynamic tracking.

When these droplets enter the region of interest (ROI) within the sorting area, they are classified and counted. If a droplet meets the predefined target criteria, its prediction result is sent via a USB (COM5) connection to an FPGA. The FPGA generates a trigger signal that produces a digital signal output, which is forwarded to an external DA mod-

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ule. This module converts the signal into a continuous 50 ms AC pulse (10 kHz sine wave). The pulse is then amplified 200-fold by a high-voltage amplifier (ATA2161) and delivered via wires to the saturated saline electrodes on the chip, thereby generating a DEP force that directs the target droplets into the collection channel, as shown in Figure 4.

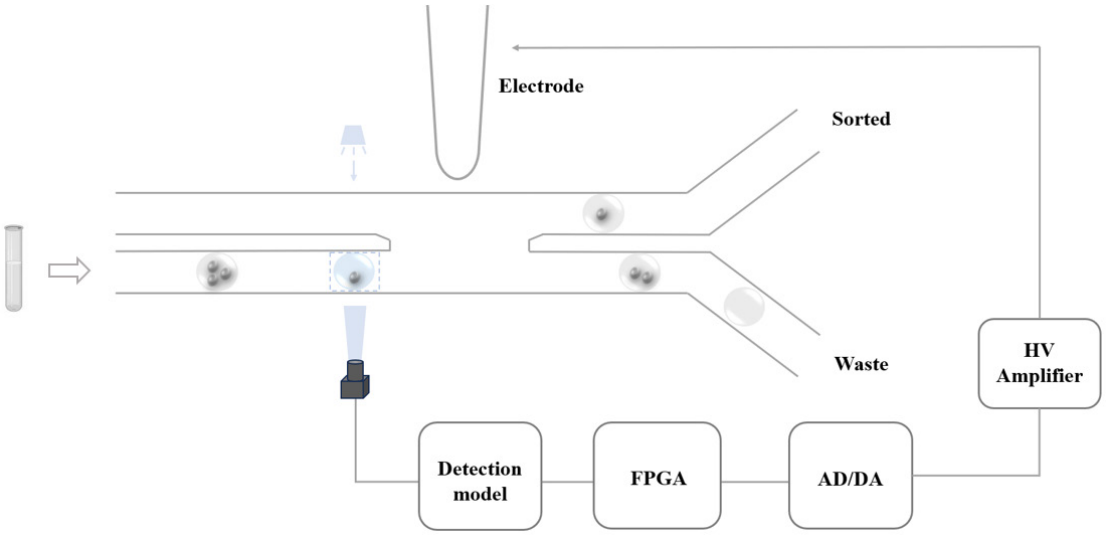

Figure 3. Sorting system.

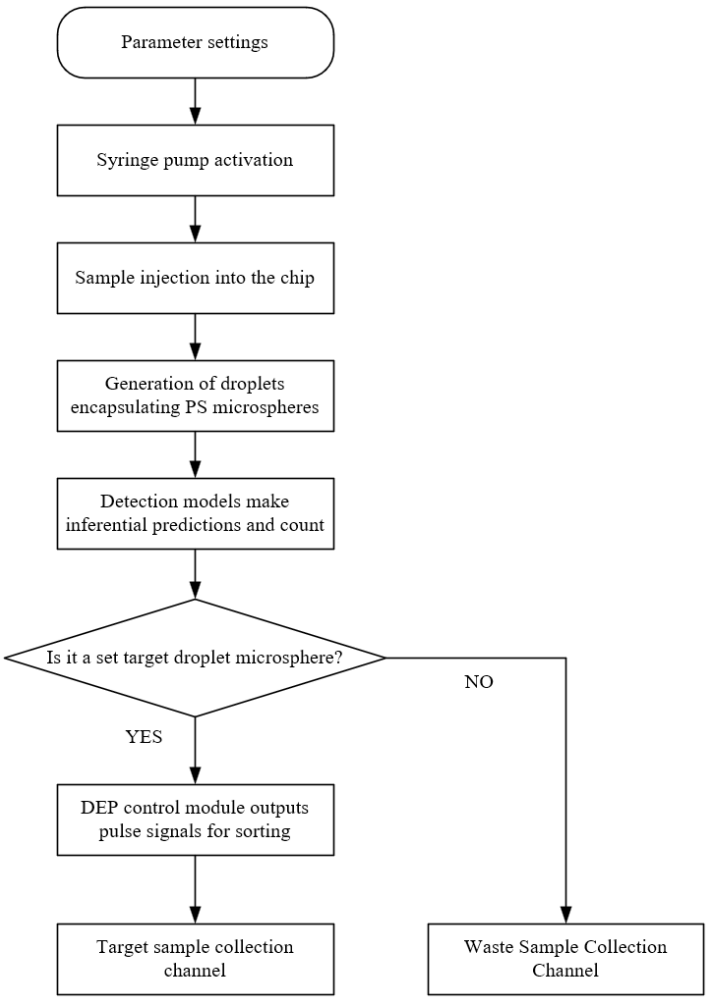

Figure 4. Sorting process.

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3.2 Real-time classification and counting of droplets

The system employs the YOLOv8 object detection algorithm and BoTSORT object tracking algorithm to perform real-time detection and counting of droplets within the sorting region. First, using the established platform, a mixture of polystyrene microsphere solution and HFE-7500 droplet generation oil containing 2% surfactant is introduced into the microfluidic chip. The chip is then placed on the stage of an inverted microscope, aligning its sorting region with the microscope's field of view. The inverted CCD camera operates at a total magnification of 10×, with an image resolution of 1280×800 pixels and a frame rate of 60 fps. The camera is connected to a computer via a USB cable to capture images and videos of droplets containing polystyrene microspheres in the sorting region. Once stable droplet generation is achieved, the detection model is activated for real-time droplet monitoring. As illustrated in Figure 5a, the system demonstrates precise identification and classification of droplets containing varying numbers of polystyrene microspheres entering the sorting region.

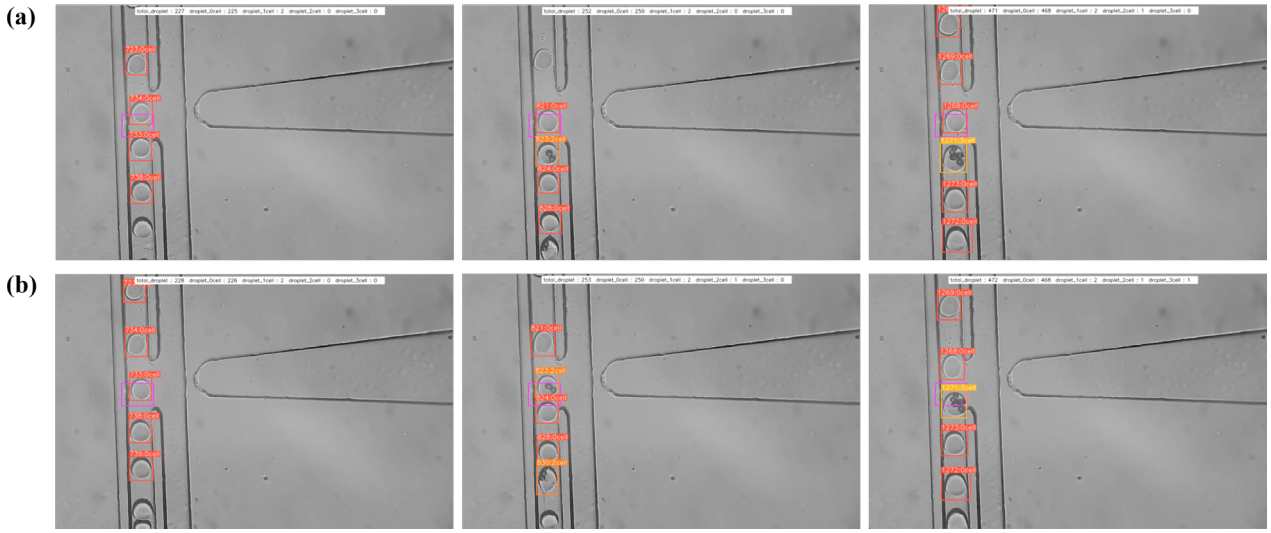

Figure 5. Real-time classification and counting of microdroplet categories; (a) Pre-ROI phase - microdroplets approaching detection zone; (b) Post-ROI phase - categorical enumeration triggered upon ROI (Region of Interest) entry with targeted sorting.

A custom program is used to define the ROI (Region of Interest), and droplets passing through this area are classified, confirmed, and counted. As shown in Figure 5b, after droplets enter the defined ROI, they are classified and counted in real-time based on the model's predicted classification results and IDs, with the counts displayed above the image field. The variable total_droplet represents the total number of droplets counted, droplet_0cell represents the count of empty droplets, droplet_1cell represents the count of droplets containing exactly one polystyrene microsphere, droplet_2cell represents the count of droplets containing exactly two polystyrene microspheres, and droplet_3cell represents the count of droplets containing three or more polystyrene microspheres. Experimental testing has demonstrated the feasibility of using the YOLOV8 object detection algorithm and the BoTSORT object tracking algorithm for real-time droplet classification prediction and counting.

3.3 Sorting and counting of individual polystyrene microsphere droplets

We conducted sorting and counting tests on droplets encapsulating single polystyrene microspheres. First, a prepared polystyrene microsphere solution and HFE-7500 droplet generation oil containing 2% surfactant were introduced into the microfluidic chip. Once stable generation of droplets encapsulating polystyrene microspheres was achieved, the YOLOv8 object detection and BoTSORT tracking algorithms were employed to perform real-time classification and counting of droplets containing single polystyrene microspheres on the chip. The real-time sorting and counting process is illustrated in Figure 6.

As shown in Figure 6a, when droplets enter the sorting region, the YOLOv8 and BoTSORT algorithms perform real-time classification predictions. Subsequently, as the droplets flow into the predefined region of interest (ROI), they are counted and categorized based on their predicted

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classes. If a droplet is identified as containing a single polystyrene microsphere (the target class), the electrodes are activated to direct the target droplet into the collection channel (Figure 6b). The sorted single-microsphere droplets are then transported through the collection channel to the outlet for retrieval (Figure 6c).

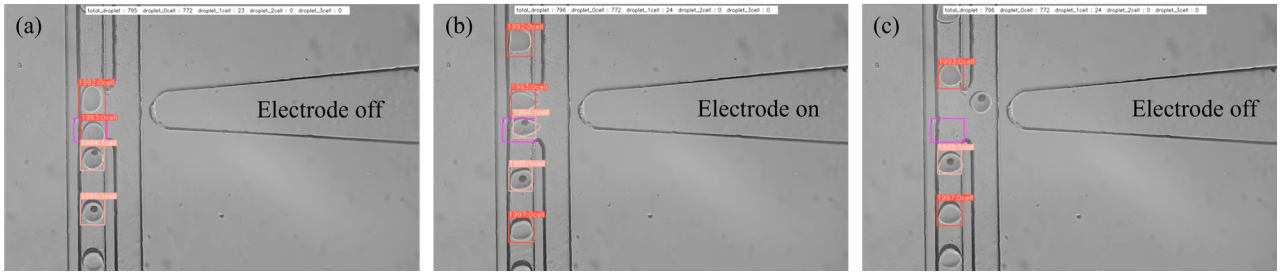

Figure 6. Sorting and counting of single polystyrene (PS) microsphere-containing droplets; (a) Real-time classification prediction of droplets entering the sorting region; (b) Target droplets flow into the region of interest (ROI), where counting is performed and electrodes are activated; (c) Sorted droplets are collected.

4. Conclusion

This study proposes, for the first time, a droplet microfluidic sorting and counting system integrating object detection and tracking algorithms. A detection model was initially developed using a dataset of polystyrene microspheres. Experimental validation was then conducted to evaluate the real-time classification and counting capabilities of the YOLOv8 detection algorithm and BoTSORT tracking algorithm for droplets. The results demonstrate that the constructed model can accurately classify and count distinct droplet types in real time by leveraging feature differences between droplets. Finally, real-time sorting and counting of droplets containing single polystyrene microspheres were performed, confirming the system's reliability. Compared to traditional labeling methods, this approach achieves high detection accuracy while eliminating cellular labeling interference, offering greater flexibility and application potential. This work provides critical technical support and a novel research platform for cutting-edge fields such as precision medicine, single-cell sequencing, and cell therapy.

References

[1] Aharon, N., Orfaig, R., & Bobrovsky, B.-Z. (2022). Bot-sort: Robust associations multi-pedestrian tracking. arXiv preprint arXiv:2206.14651. https://doi.org/10.48550/arXiv.2206.14651

[2] Gardner, K., Uddin, M. M., Tran, L., Pham, T., Vanapalli, S., & Li, W. (2022). Deep learning detector for high precision monitoring of cell encapsulation statistics in microfluidic droplets. Lab on a Chip, 22(21), 4067-4080. https://doi.org/10.1039/d2lc00462c

[3] Hochstetter, A., Vernekar, R., Austin, R. H., Becker, H., Beech, J. P., Fedosov, D. A., Gompper, G., Kim, S.-C., Smith, J. T., Stolovitzky, G., Tegenfeldt, J. O., Wunsch, B. H., Zeming, K. K., Krüger, T., & Inglis, D. W. (2020). Deterministic Lateral Displacement: Challenges and Perspectives. ACS Nano, 14(9), 10784-10795. https://doi.org/10.1021/acsnano.0c05186

[4] Howell, L., Anagnostidis, V., & Gielen, F. (2021). Multi-Object Detector YOLOv4-Tiny Enables High-Throughput Combinatorial and Spatially-Resolved Sorting of Cells in Microdroplets. Advanced Materials Technologies, 7(5). https://doi.org/10.1002/admt.202101053

[5] https://github.com/ultralytics/ultralytics

[6] Kemna, E. W. M., Schoeman, R. M., Wolbers, F., Vermes, I., Weitz, D. A., & van den Berg, A. (2012). High-yield cell ordering and deterministic cell-in-droplet encapsulation using Dean flow in a curved microchannel. Lab on a Chip, 12(16). https://doi.org/10.1039/c2lc00013j

[7] Link, A., McGrath, J. S., Zaimagaoglu, M., & Franke, T. (2022). Active single cell encapsulation using SAW overcoming the limitations of Poisson distribution. Lab on a Chip, 22(1), 193-200. https://doi.org/10.1039/d1lc00880c

[8] Tang, R., Xia, L., Gutierrez, B., Gagne, I., Munoz, A., Eribez, K., Jagnandan, N., Chen, X., Zhang, Z., Waller, L., Alaynick, W., Cho, S. H., An, C., & Lo, Y.-H. (2023). Low-latency label-free image-activated cell sorting using fast deep learning and AI inferencing. Biosensors and Bioelectronics, 220. https://doi.org/10.1016/j.bios.2022.114865

[9] Xi, H.-D., Zheng, H., Guo, W., Gañán-Calvo, A. M., Ai, Y., Tsao, C.-W., Zhou, J., Li, W., Huang, Y., Nguyen, N.-T., & Tan, S. H. (2017). Active droplet sorting in microfluidics: a review. Lab on a Chip, 17(5), 751-771. https://doi.org/10.1039/c6lc01435f

[10] Zhong, J., Liang, M., Tang, Q., & Ai, Y. (2023). Selectable encapsulated cell quantity in droplets via label-free electrical screening and impedance-activated sorting. Materials Today Bio, 19. https://doi.org/10.1016/j.mtbio.2023.100594

 DOI: https://doi.org/10.26549/met.v8i1.23688