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Cell Classification Based on Artificial Intelligence Analysis of Cell Images in Microfluidic Chip †

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Abstract: We developed a low-cost, multi-classification, label-free and high-precision method for cell classification, which combines microfluidic technology with a deep learning algorithm. The recognition of the states of red blood cells was selected as a typical example to demonstrate the feasibility of the method. The microfluidic channel is designed to effectively and controllably solve the problem of cell overlap, which has a severe negative impact on the identification of cells. The object detection model based on YOLOv4 is optimized and used to recognize multiple RBCs simultaneously in the whole field of view, so as to classify them into different morphological subcategories and count the numbers in each subgroup.

Keywords: cell classification; artificial intelligence; object detection model; microfluidic chip

1. Introduction

Biological cells are micro-particles with a certain size, shape and state. Most biological cells have a complex, irregular appearance and internal structure, organic or chemical composition and biological activity. Taking the red blood cell (RBC) as an example, it has a double concave disk shape with a smooth edge. During the storage of blood, RBCs undergo reversible deformation from a concave smooth disk to a wrinkled disk shape (with slight and severe wrinkles on the edges), and a spiny ball shape (needle shaped sphere), and finally they change to an irreversible spherical shape (wrinkle ball and smooth ball) [1]. The quality of stored blood (or RBCs) can be assessed by the number of cells with different morphologies. Therefore, it is of high demand to classify cells into subcategories using a simple but reliable method.

Traditionally, the morphology of RBCs was characterized using an optical microscope. However, the preparation of the blood smear involved multiple steps and complex operations. The cell classification required an experienced person and the subjective factors had significant impacts. With the development of automatic cell detection instruments, image flow cytometry started to be applied for cell classification [2]. It has the advantages of fast speed, high throughput and a high degree of automation. However, the cost and requirement for an efficiency image processing program limited its wide applications. Artificial intelligence, especially the deep learning algorithm, is a new technology in the field of image processing, which has attracted a lot of attention in recent years. To improve the cell classification efficiency of image flow cytometry, Doan et al. demonstrated the use of deep learning to characterize RBC lesions [3]. Their trained neural network achieved 76.7% agreement with experts in classifying seven clinically relevant RBC morphologies, which was comparable between different experts. Zheng et al. developed a simple method for the

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characterization of RBC deformability during blood storage by combining a microfluidic device with a high speed camera [4]. Cells were introduced in the microchannel with a stable flow field and the deformation behavior was recorded. The cell quality was then assessed by the time constants and circularity distribution widths. Wang et al. reported the use of Fourier ptychographic microscopy and the SO-YOLO algorithm for the detection of white blood cells [5]. The new microscope yielded high resolution, wide field-of-view blood cell images at one time and the SO-YOLO algorithm had higher accuracy and speed compared to other deep learning algorithms. The above literature proved the feasibility of artificial intelligence in cell classification. The establishment of a low-cost, multi-classification, high-precision and good adaptability system is still in high demand for applications.

Herein, a microfluidic system combined with an object detection algorithm is reported. The microfluidic chip is used to solve the problems of cell overlap and aggregation, which have a severe negative impact on cell identification and classification. The optimized object detection algorithm can identify all the cells in the whole field of view, achieve classification into multiple categories and automatically count the numbers. The quality of blood can be assessed quantitatively by the number of cells in each subgroup.

2. Materials and Methods

2.1. Preparation of Microfluidic Chip

The microfluidic chip with a PDMS channel and glass substrate was fabricated using standard photolithography and soft lithography [6]. The chip structure was designed by autoCAD and printed on a plastic mask. A spin coating of SU8 2010 was first conducted to obtain a thin layer of photoresist with a thickness of 12 μm on the cleaned silicon wafer with a diameter of 4 inches, followed by soft bake at 65 °C for 5 min and hard bake at 95 °C for 10 min. Exposure was then conducted using mask aligner URE2000/35 and post bake at 95 °C for 5 min and developing were performed to obtain the channel mold. The PDMS pre-polymer and curing agent was mixed with a mass ratio of 10:1 and poured onto the fluorosilane passivated mold. The replicated channel was then obtained and bonded to a clean glass slide using a plasma cleaner.

2.2. Acquisition and Labeling of RBC Images

The blood samples containing red blood cells were provided by the Chongqing University Cancer Hospital from a physical examination of healthy people. The blood was diluted 500 times using pH 7.4 buffer solution before it was introduced into a microfluidic chip.

Images of cells were captured using the camera mounted on the optical microscope when the sample was introduced into the microfluidic chip. The magnification of the microscope was set to ×400 to obtain clear images of cells. The recoded images were divided into the training set, the verification set and the test set. All the cells were classified into six categories: smooth disc (SDC), crenated disc (CDC), crenated discoid (CDD), crenated spheroid (CSD), crenated sphere (CSE) and smooth sphere (SSE).

2.3. Construction and Training of Object Detection Model

There are many object detection models in deep learning and the YOLOv4 model is one of the best models in terms of detection speed. The core idea of the YOLO model is to simplify the object detection problem into a regression problem, starting directly from the pixels of the image, to obtain the object detection frame and its classification probability.

To construct the model, the input image, a feature extraction network, a Neck network and the output have to be implemented. The size of the recorded image was unified to 416 × 416 × 3 as the input and data enhancement methods such as brightness enhancement, contrast enhancement and sharpness enhancement were conducted. Ghostnet was selected as the feature extraction network and the Mish activation function and the Dropblock module were used to further increase the generalization ability of the model. The Neck network can be used to further enhance the diversity and robustness of features. The SPP (spatial pyramid pooling) module was used to fuse feature maps of different scales of cell

images, and the top-down FPN feature pyramid and bottom-up PAN feature pyramid were used to improve the feature extraction ability of cell images. The number of cells in each category was set as the output of the model.

3. Results

3.1. The Setup and the Designed Microfluidic Chip

The microfluidic chip with a single channel was designed (Figure 1a). The channel length, width and height were 2 cm, 100 μm and 12 μm, respectively. The low height of the channel and large dilution factor for blood eliminated the overlap and aggregation phenomenon in the channel and the top view of the RBC could be recorded. The setup for the whole detection system is shown in Figure 1b. The syringe pump was used for sample introduction. The microfluidic chip was placed on the microscope stage and a high speed camera was mounted to capture the clear image of cells. The images were processed using the developed object detection algorithm stalled on the computer. The whole system had a similar function to the image flow cytometry. Cells were traveling in the microfluidic chip and the confined space ensured that all the images were of the top-view of cells. The analyses of the captured images were processed automatically using the computer.

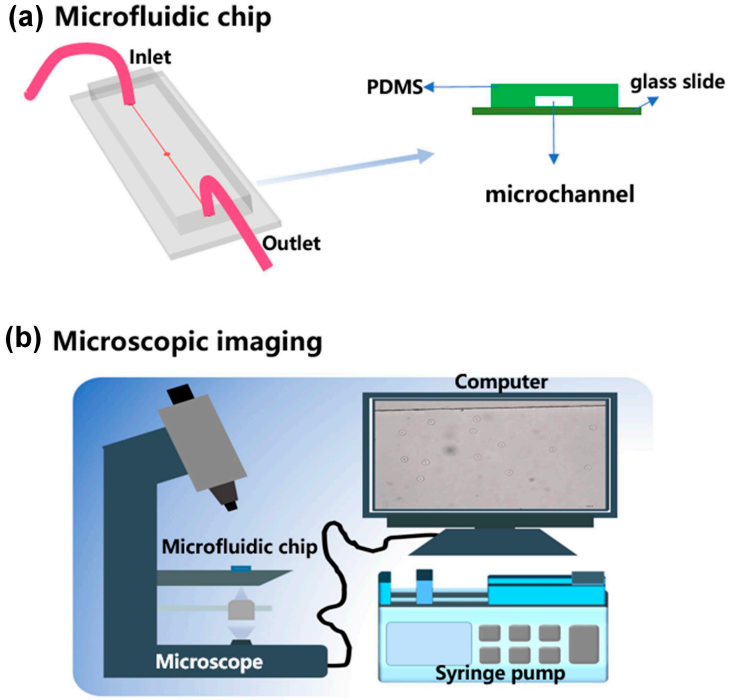

Figure 1. (**a**) The designed microfluidic chip; (**b**) the setup for cell imaging and analyzing.

3.2. Identification and Classification of RBCs

In order to verify the effectiveness of the developed object detection algorithm based on YOLOv4, blood samples stored for 1 week and 6 weeks were prepared and tested. Figure 2 showed the identification and classification of RBCs in the microchannels. All the RBCs could be detected successfully and they were classified into different subgroups. RBCs would transform sequentially from SCD to CDC, CDD, CSD, CSE and SSE during storage. Therefore, more cells with CSE and SSE morphologies represented a longer storage of blood when comparing Figure 2b to Figure 2a. There were 12 normal RBCs (SDC) and 1 CDD cell in the whole imaging area (Figure 2a), while there were 2 CSD cells, 9 CSE cells and 10 SSE cells, indicating the severe degradation of RBCs after storage for 6 weeks. The results successfully demonstrated that the developed method can be used for cell classification based on morphology differences.

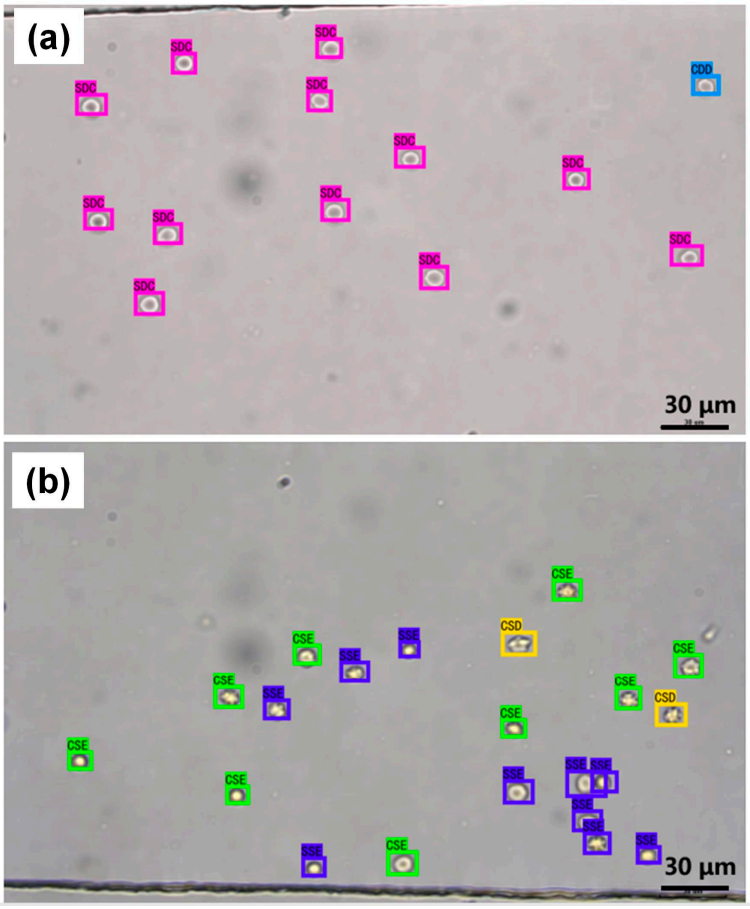

Figure 2. The RBC identification and classification using the developed object detection algorithm. (**a**) Images of cells from blood stored for 1 week; (**b**) images of cells from blood stored for 6 weeks.

4. Discussions

At present, the classification of biological cells includes traditional optical microscope, flow cytometry after fluorescent straining and image flow cytometry, etc. However, these methods suffer several drawbacks such as low sensitivity, expensive and bulky instruments and/or complex processes. With the rapid development of computer algorithms, artificial intelligence (AI) has started to be applied in cell classification, which makes it simple and removes the need for any labeling reagent. The main problems of cell classification based on AI are focused on the influence of image background, cell aggregation, small size and the lack of enough training samples. A combination of microfluidic technology with deep learning algorithms was applied for cell identification and classification.

The evaluation of red blood cell quality is significant before blood transfusion in clinical treatment. Our work presented a low-cost, multi-classification, high-precision and intelligent method for evaluating RBC quality. A microfluidic chip was used to avoid negative effects in detection and recognition such as cell overlap and aggregation. Based on the target detection model YOLOv4, network structure optimization was carried out to achieve the accurate detection and classification of RBCs simultaneously in the whole imaging area. This method has the merit of providing cell identification in a wide channel, with no need for single cell alignment, as for the image cytometry, and it has potential applications for the classification of biological cells with different morphologies.

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Institutional Review Board Statement: The study was conducted according to the guidelines—Interim Measures on the Administration of Human Biological Samples for Scientific Research in Medical and Health Institutions, and the ethical justification was conducted by the Affiliated Chongqing University Cancer Hospital Ethics Committee with the ethical code: CZLS2021267-A.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are openly available in https://github.com/wwwyyp/CellClassification_in_MicrofluidicChip.

Conflicts of Interest: The authors declare no conflict of interest.

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