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# Deep Learning Assisted Microfluidic Impedance Flow Cytometry for Label-free Foodborne Bacteria Analysis and Classification *

Shuaihua Zhang, Ziyu Han, Zhe Feng, Meiqing Sun and Xuexin Duan

*Abstract*— **According to the urgent need for rapid detection and identification of foodborne bacteria to prevent public health event, a microfluidic electrical impedance flow cytometry assisted with convolutional neural network (ConvNet) based deep learning algorithm was proposed in this study to analyze the impedance signals of bacteria. With the assistance of the deep learning algorithm, *Escherichia coli* (EPEC), *Salmonella enteritidis* (SE) and *Vibrio parahaemolyticus* (VP) were identified with an accuracy of 100%. The proposed impedance based analysis system can be potentially applied for pre-classification of different subtypes of bacteria in a label-free manner.**

*Clinical Relevance*—**The whole platform can be miniaturized and applied for point-of-care testing (POCT) of pathogenic bacteria detection.**

## I. Introduction

According to the large number of illness every year due to infections of foodborne pathogens [1-3], detection and classification of pathogenic bacteria play important role in food safety monitoring [4] and clinical treatment [5-6]. Conventional bacteria detection methods include plate culturing in selective medium [7], mass spectrometry (MS) [8], enzyme linked immunosorbent assay (ELISA) [9] and polymerase chain reaction (PCR) [10]. Recently, benefiting from the rapid progress of microfluidic technology, a variety of novel biosensors were developed to improve the accuracy and efficiency of bacteria detection including optical [11] and electrical [12] methods.

Microfluidic impedance flow cytometry (IFC) [13-14], a major branch of electrical biosensors, has emerged as a label-free, low-cost, high-throughput method for rapid particle sizing [15] and cell analysis [16]. When cells pass through the sensing zone, the resistance between the electrodes changes accordingly, resulting in electrical pulses related to the size and other dielectric parameters of cells. However, it is still challenging to process and analyze the huge amount of impedance data (including time domain and frequency domain), especially for bacteria classification applications. Therefore, an accurate and rapid data analysis method is urgently needed.

Deep learning, as a representative algorithm of artificial intelligence (AI) [17] and an unsupervised learning model, has been applied for big data analysis and unknown sample identification based on the weight value of neurons in all network layers. Due to the high accuracy and efficiency for data processing, studies have been carried out combining deep learning with microfluidic systems for complex sample classification [18-19].

In this paper, a microfluidic impedance flow cytometry was proposed for *Escherichia coli* (EPEC, CICC 10664), *Salmonella enteritidis* (SE, CICC 21513) and *Vibrio parahaemolyticus* (VP, CICC 21617) analysis (All biological experiments were carried out in a BSL-2 laboratory of Wuqing District Center for Disease Control and Prevention, Tianjin, China). The impedance data of EPEC, SE and VP was analyzed and classified using a ConvNet deep learning algorithm. After designing and training the ConvNet model with randomly picked impedance data of three types of foodborne bacteria, the discrimination accuracy was 96% for the training data and the classification accuracy was 100% for the unlabeled data testing. With the assistance of the deep learning algorithm, the proposed system can be potentially applied for pre-analysis of different bacteria subtypes by building impedance databases obtained from various bacteria collected by microfluidic chip.

## II. Experimental Setup

The schematic diagram of the proposed the microfluidic electrical impedance flow cytometry system is shown in Figure 1a. The microfluidic chip consists of a microchannel, which is 15 μm in width and 9.4 μm in height. A three-electrodes sensing strategy was employed with the electrode width of 15 μm and the spacing of 15 μm. When an AC signal was applied to the middle electrode, the differential current was pre-amplified 1000 times by a pre-current amplifier (Zurich Instruments HF2TA, Switzerland) and then measured by an impedance analyzer (Zurich Instruments HF2IS, Switzerland). When bacteria pass above the sensing electrodes, symmetrical characteristic peaks can be recorded by the impedance analyzer for further data analysis (Figure 1b).

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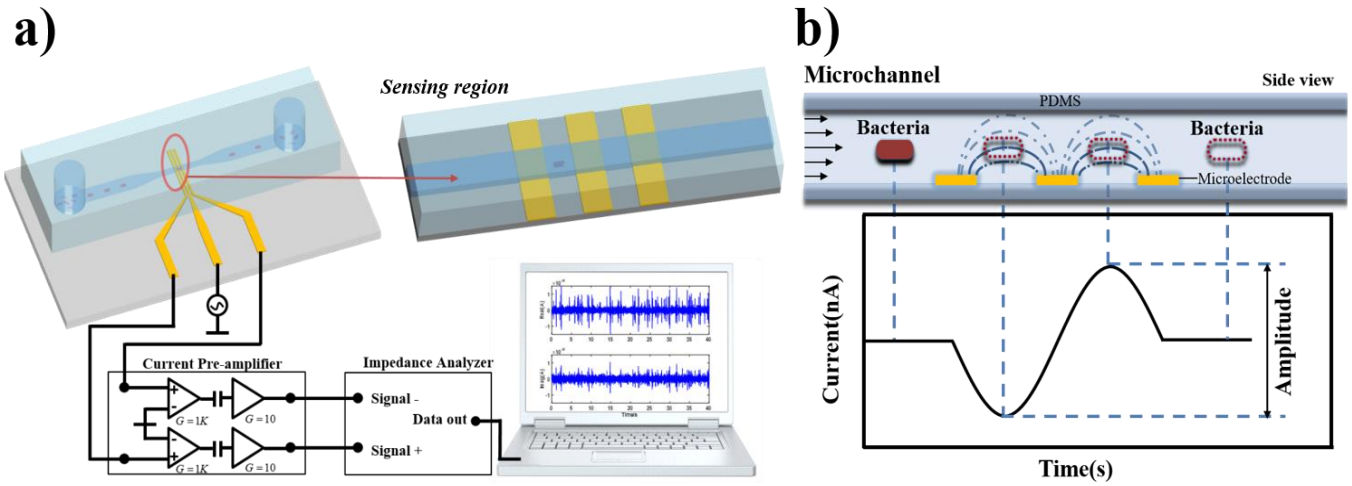


Figure 1. The schematic diagram of a) the microfluidic impedance flow cytometry device and system for bacteria analysis and b) the characteristic peak signal of single bacteria.

## III. RESULTS AND DISCUSSION

In order to test the feasibility of the proposed system for bacteria sensing, bacteria counting was carried out by measuring bacteria suspensions with a series of concentrations. The real-time differential current signal was measured under the excitation voltage of 3 V/2.5 MHz and the pneumatic driving pressure of 100 mbar. PBS solution was used to clean the channel for 20 minutes at 345 mbar pressure to remove the residual bacteria of previous test. Each test was repeated 3 times. Figure 2a shows the real-time signal of bacteria suspensions with increasing concentrations ranging from 42 $\pm$ 9 CFU/ul to 22441 $\pm$ 135 CFU/ul that calculated by Equation (1). As the increment of bacterial concentration, the number of the electrical peaks increases accordingly with a linear relation to bacteria concentration, as shown in Figure 2b.

$$V = \frac{N * T}{L * W * H * t} \tag{1}$$

Here, N, T and t refer to the number of peak signals, the time interval between the positive and negative pulse, and the acquisition time, respectively.

Due to the differences in the shape, size and other biophysical characteristics between various types of bacteria, the electrical properties vary accordingly. To make sure that the bacteria pass above the sensing electrodes one by one, the stock solution was diluted with 10 mM PBS solution to an optimal concentration of $2\times10^5$ CFU/ml. Figure 3 shows the scatter plots of amplitude against phase diagrams of three types of bacteria (EPEC, SE and VP) under 2 V excitation voltage comprising six different excitation frequencies (100 kHz, 1 MHz, 2 MHz, 3 MHz, 5 MHz, 8 MHz) with 5 mbar pneumatic driving pressure. It is worth noting that, under particular frequency, different distributions were obtained, yet obvious overlaps between different types still occurred. Therefore, it is difficult to directly discriminate different bacteria by simply analyzing the impedance of bacteria under a single frequency.

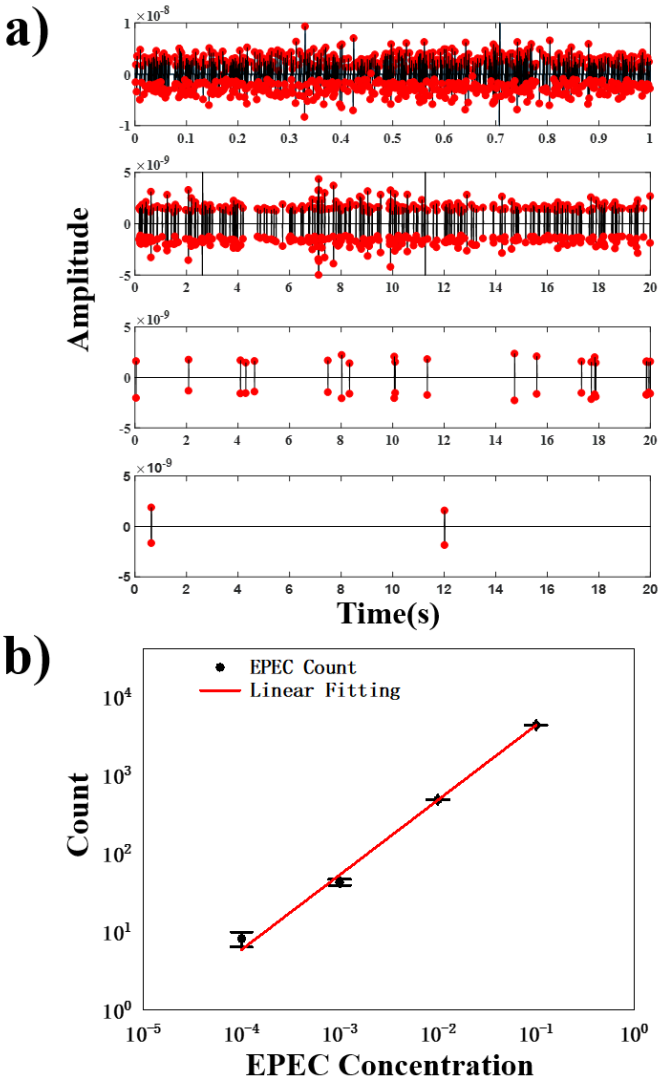


Figure 2. a) The real-time signal of four concentrations of EPEC samples. b) The linear relation between the number of peaks and the concentration of EPEC.

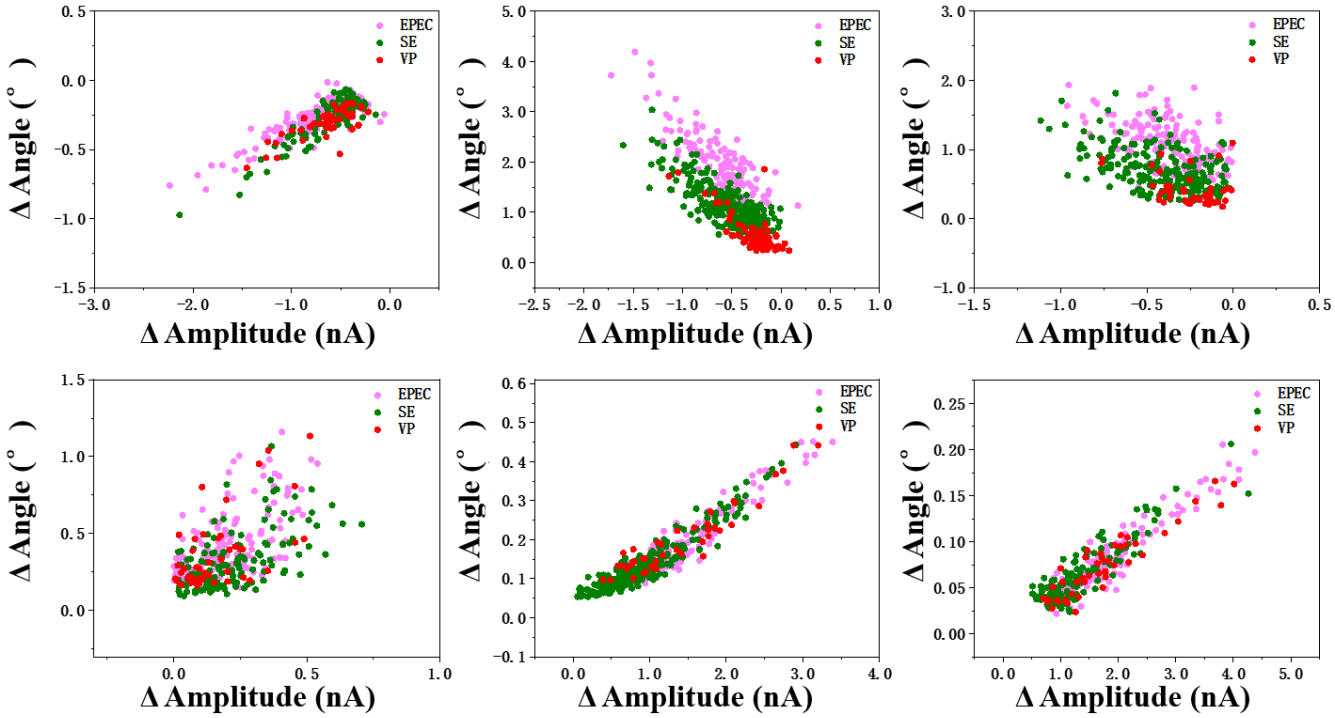


Figure 3. The scatter plots of amplitude vs. phase angle of three types of bacteria under excitation frequencies of 100 kHz, 1 MHz, 2 MHz, 3 MHz, 5 MHz and 8 MHz.

To improve the performance of bacteria discrimination and classification, a ConvNet algorithm, as a representative algorithm of deep learning, was employed to process the impedance data of different bacteria. As shown in Figure 4, the impedance data was transformed into three-dimensional matrix data to match the algorithm. The proposed ConvNet model includes an input layer, a padding layer, two convolution layers, a full connection layer and an output layer. The convolution depth was set as 8 layers, and the rectified linear unit (ReLU) was used as the activation function. ReLU is to complete the nonlinear transformation of data and solve the problem of insufficient classification ability of linear model. To prevent over fitting phenomenon, a dropout algorithm was set 0.2 and used in both convolution layers to optimize the number of unnecessary neurons.

For each type of bacteria, the impedance data was randomly divided into two groups, including the training data and the testing data (about 450 bacteria for training and 150 bacteria for testing). The training data was then input into the ConvNet network for model training. After the model parameters were readily obtained, the test data was input into the trained network model to verify the reliability and accuracy. As shown in Figure 5a, the accuracy of the training result is above 96%, and the prediction accuracy of bacteria discrimination is 100% under the excitation frequency of 1 MHz, 2 MHz, 3 MHz and 5 MHz. For the purpose to show the advantages of ConvNet deep learning algorithm in bacteria classification combined with impedance data, we further input the same identical data into the support vector machine (SVM) classification model. As for SVM algorithm,

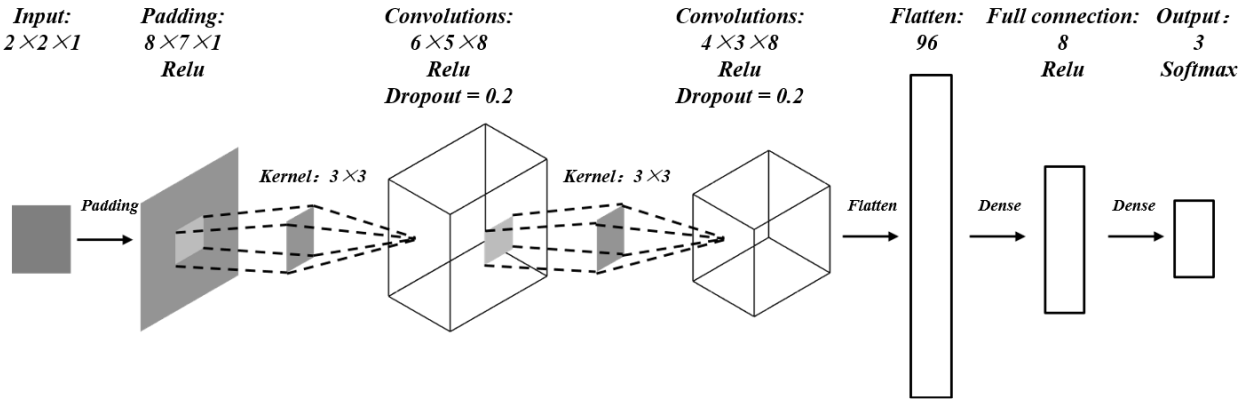


Figure 4. The structure of convolutional neural network (ConvNet) model for bacteria classification, including an input layer, a padding layer, two convolution layers, a full connection layer and an output layer.

the accuracy of bacteria classification of the training data is around 60%, which is much lower that of the ConvNet algorithm. Figure 5b shows the confusion matrix of the prediction accuracy of the test data of the three types of bacteria under 1 MHz. It is worth noting that the prediction accuracy of the three types of bacteria are 100%, revealing the advantage and reliability of the ConvNet deep learning network assisted microfluidic impedance flow cytometry for bacteria classification.

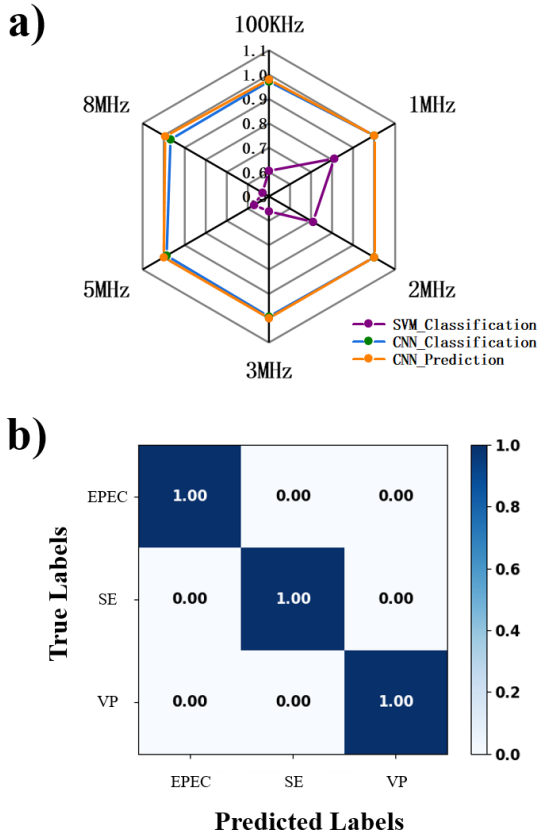


Figure 5. a) Comparison between ConvNet algorithm and SVM algorithm. b) The confusion matrix of the bacteria prediction accuracies using the trained ConvNet model under 1M Hz.

## IV. Conclusions

In this work, a deep learning assisted microfluidic impedance flow cytometry was developed for foodborne bacteria analysis. By employing the ConvNet algorithm of data analyzing, *Escherichia coli*, *Salmonella enteritidis* and *Vibrio parahaemolyticus* were distinguished with a 100% accuracy. This study provides a novel method for data analysis of impedance measurement, which can be applied as a complementary method for bacteria phenotyping studies. Furthermore, the proposed method and system can be further miniaturized for miniaturize for point-of-care testing (POCT) of foodborne bacteria in a rapid and label-free manner.

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